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ABSTRACT

This is one of several short-term courses developed to assist in the training of waste water treatment plant operational personnel in the tests, measurements, and report preparation required for compliance with their NPDES Permits. The Student Reference Manual provides step-by-step procedures for laboratory application of equipment operating procedures for effluent monitoring. Each lesson outlines a specific objective, description of the analysis, and the applicability of the procedure. Parameters of this course include BoD, pH, fecal coliform, residual chlorine, suspended solids, and open channel flow. (CS)

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Self-Monitoring Procedures: Basic Parameters for Municipal Effluents

ED147195

STUDENT REFERENCE MANUAL

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF WATER PROGRAM OPERATIONS

SE 023 383

SELF-MONITORING PROCEDURES: BASIC PARAMETERS FOR MUNICIPAL EFFLUENTS

This course is designed for the treatment plant operator or technician who is required to monitor effluent discharges under a National Pollutant Discharge Elimination System (NPDES) Permit, and who has had little or no previous experience in wastewater analysis or flow measurement.

Parameters included in this course are BOD_5, pH, Fecal Coliform, Residual Chlorine, Suspended Solids, and Open Channel Flow. At the conclusion of this training, the student will be familiar with the standard test procedure for each parameter, will have performed each analysis, and will be able to use a parshall flume or weir to measure effluent flow. He will also know what equipment and supplies are needed in connection with each procedure.

U. S. ENVIRONMENTAL PROTECTION AGENCY
Office of Water Program Operations
National Training and Operational Technology Center

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CONTENTS

Title or Description	Outline Number
Determination of Five-Day Biochemical Oxygen Demand (BOD_5)	1
Winkler Determination of Dissolved Oxygen-Azide Modification	2
Dechlorination of Samples for Biochemical Oxygen Demand and Seeding of the Dilution Water	3
Determination of Dissolved Oxygen Using A Dissolved Oxygen Meter	4
Determination of Dissolved Oxygen in Wastewater: Polarographic Probe Method	5
pH Determination of Wastewater and Wastewater Treatment Plant Effluents	6
Collection and Handling of Bacteriological Samples from a Wastewater Treatment Facility	7
Fecal Coliform Test by the Multiple Dilution Tube Method	8
Fecal Coliform Test by the Membrane Filter Method	9
Calculation of the Geometric Mean of Coliform Counts by the Use of Logarithms	10
Measurement of Flow in an Open Channel by Parshall Flume	11
Measurement of Flow in an Open Channel by Sharp-Crested Weir	12
Amperometric Determination of Free and Combined Residual Chlorine in Water	13
Amperometric Determination of Total Residual Chlorine in Wastewater	14
Titrimetric Determination of Total Residual Chlorine in Wastewater Effluents	15
Determination of Total Suspended (Non-Filterable) Solids, mg/liter	16
Settleable Solids, mg/liter (Imhoff Settling Cone)	17
Reporting of Self-Monitoring Data	18

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

DETERMINATION OF FIVE-DAY BIOCHEMICAL OXYGEN DEMAND (BOD_5)

as applied in

WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

Developed by the

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

EFFLUENT MONITORING PROCEDURE: Determination of Five-day Biochemical Oxygen Demand (BOD_5)

This operational procedure was developed by:

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EFFLUENT MONITORING PROCEDURE: Determination of Five-day Biochemical Oxygen Demand (BOD_5)

1. Analysis Objectives:

   The learner will determine the five-day biochemical oxygen demand of a sewage sample.

2. Brief Description of Analysis:

   The sample is diluted with a high quality distilled water containing nutrient salts and a buffer. Two biochemical oxygen demand (BOD) bottles are filled with the diluted sample. The dissolved oxygen (DO) content of the first bottle is determined, and expressed as mg of DO/liter. The second bottle is stored in the dark at 20°C for five days. During the five-day period, microorganisms in the sample break down complex organic matter in the sample, using up oxygen in the process. At the end of the five-day period, the DO content of the second BOD bottle is determined, and again expressed as mg of DO/liter. The depletion in oxygen content, divided by the percent of sample used (expressed as a decimal fraction) is the five-day biochemical oxygen demand expressed as milligrams of BOD per liter of sample. BOD_5 is the symbol for the five-day biochemical oxygen demand.

3. Applicability of this Procedure:

   This effluent monitoring procedure describes the determination of five-day biochemical oxygen demand. It is not applicable when:

   a. The sample contains caustic alkalinity or acidity.

   b. The sample contains residual chlorine compounds. (In this case, consult the Effluent Monitoring Procedure on the Dechlorination of Samples for Biochemical Oxygen Demand and Seeding of the Dilution Water. It covers only the dechlorination and seeding aspects of the biochemical oxygen demand determination, and then refers the reader back to this Effluent Monitoring Procedure.)

   c. The sample contains other toxic substances such as those contained in plating wastes.

   d. The sample is supersaturated with oxygen.

This procedure was excerpted from Standard Methods for the Examination of Water and Wastewater, 14th ed., pp. 543-550, 1975.

EFFLUENT MONITORING PROCEDURE: Determination of Five-day Biochemical Oxygen Demand (BOD_5)

Equipment and Supply Requirements

A. Capital Equipment:

1. Trip balance, 100 g. capacity
2. Still, or other source of distilled water
3. Incubator capable of maintaining a temperature of $20^{\circ}C \pm 1^{\circ}C$, and large enough to hold four 300 ml BOD bottles and a 3 liter jug or bottle

B. Reusable Supplies:

1. Brushes (for cleaning glassware)
2. Brush (for cleaning balance)
3. Laboratory apron
4. Safety glasses
5. One spatula (medium size)
6. One distilled water plastic squeeze bottle
7. One pen or pencil
8. One notebook (for recording data)
9. Seven plastic weighing boats (2-3 inches square)
10. Sponges (for cleaning of laboratory table tops)
11. One 3 liter jug or bottle with narrow neck
12. One powder funnel, about 3 inch diameter
13. One 1 liter volumetric flask
14. Four 1 liter glass-stoppered bottles
15. One 1 liter graduated cylinder
16. One 2 liter graduated cylinder
17. One siphon (long enough for use with the 2 liter graduated cylinder)
18. Four 1 ml volumetric pipets
19. One 10 ml volumetric pipet
20. One 20 ml volumetric pipet
21. One plunger type mixer (for use with the 1 liter graduated cylinder)
22. Four 300 ml (± 3 ml) BOD bottles (see pages 16 and 17)
23. Equipment for doing a Winkler DO determination-azide modification, see EMP on Winkler Determination of Dissolved Oxygen-Azide Modification.
24. One dissolved oxygen meter. See the EMP on Determination of Dissolved Oxygen Using a Dissolved Oxygen Meter (a Weston & Stack, Model 300) or the EMP on Determination of Dissolved Oxygen in Wastewater: Polarographic Probe Method (a Yellow Springs, Model 54).
25. One 2 liter beaker (for preparing cleaning solution)
26. One 12 inch stirring rod (for preparing cleaning solution)

C. Consumable Supplies:

1. Small wad of cotton (to plug the 3 liter jug or bottle)
2. 8.5 g. of potassium dihydrogen phosphate, KH_2PO_4
3. 21.75 g of dipotassium hydrogen phosphate, K_2HPO_4
4. 33.4 g of disodium hydrogen phosphate heptahydrate, $Na_2HPO_4 \cdot 7H_2O$
5. 1.7 g of ammonium chloride, NH_4Cl
6. 22.5 g. of magnesium sulfate heptahydrate, $MgSO_4 \cdot 7H_2O$
7. 27.5 g. of anhydrous calcium chloride, $CaCl_2$

C. Consumable Supplies (Continued)

8. 0.25 g. of ferric chloride hexahydrate, $FeCl_3 \cdot 6H_2O$
9. Reagents for doing a Winkler DO determination-azide modification, see EMP on Winkler Determination of Dissolved Oxygen-Azide Modification
10. Reagents for use with a dissolved oxygen meter. See EMP on Determination of Dissolved Oxygen Using a Dissolved Oxygen Meter (a Weston & Stack, Model 300) or the EMP on Determination of Dissolved Oxygen in Wastewater: Polarographic Probe Method (a Yellow Springs, Model 54).
11. Concentrated sulfuric acid, H_2SO_4
12. Sodium Dichromate, $Na_2Cr_2O_7$
13. Soap

(Items 11, 12, and 13 are for cleaning glassware. The quantities needed will therefore vary.)

All reagents should be of high quality. Different chemical manufacturers may have different ways of indicating a high quality reagent. While no endorsement of one chemical manufacturer over another is intended, the following are some designations used in four chemical catalogs to indicate high quality reagents.

Catalog	Designations
Thomas	Reagent, ACS, Chemically Pure (CP)
Matheson, Coleman & Bell	Reagent, ACS
Curtin Matheson Scientific, Inc.	Primary Standard, ACS, AR
Fisher	Certified, ACS

EFFLUENT MONITORING PROCEDURE: Determination of Five-Day Biochemical Oxygen Demand(BOD_5)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Equipment Preparation 1. Cleaning of glassware	1. Clean all glassware and rinse with distilled water.		V.A.1.1 (p. 15)
2. Balance inspection	1. Check all balances for cleanliness and proper operation.	1a. Consult the manufacturer's manual supplied with the balance for assistance in correcting any malfunctions.	
B. Reagent Preparation 1. Distilled water	1. Distill 3 liters of water into a small neck jug (or large bottle).	1a. When preparing solutions, unless otherwise specified, the term water means distilled water. 1b. Unless otherwise specified, solutions should be stored in glass stoppered bottles.	
	2. Plug the jug with a loose fitting piece of cotton.	2a. Air should be able to pass freely into the jug.	
	3. Store the jug at 20°C ± 1°C for 48 hours prior to use,	3a. This length of time has been determined simply on the basis of experience.	
	4. or aerate the water just prior to use.	4a. Do this by shaking the water in a half-filled jug, 4b. or by using a clean supply of compressed air. (Be cautious about air jets and motors which may simply contaminate the water with oil).	
2. Phosphate buffer solution	1. Weigh 8.5 g. of potassium dihydrogen phosphate, KH_2PO_4.	1a. Use plastic weighing boats for weighing the solids. 1b. Use a trip balance for weighing the solids.	
	2. Weigh 21.75 g. of dipotassium hydrogen phosphate, K_2HPO_4.		
	3. Weigh 33.4 g. of disodium hydrogen phosphate heptahydrate, $Na_2HPO_4 \cdot 7H_2O$		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)	4. Weigh 1.7 g. of ammonium chloride, NH_4Cl.		
	5. Dissolve the four chemicals together in about 500 ml. of water.		
	6. Dilute to 1 liter.		
3. Magnesium Sulfate Solution	1. Dissolve 22.5 g. of magnesium sulfate heptahydrate, $MgSO_4 \cdot 7H_2O$, in water and dilute to 1 liter.		
4. Calcium Chloride Solution	1. Dissolve 27.5 g. of anhydrous calcium chloride, $CaCl_2$, in water and dilute to 1 liter.		
5. Ferric Chloride Solution	1. Dissolve 0.25 g. of ferric chloride, $FeCl_3$, in water and dilute to 1 liter.		
6. Dilution Water	1. Siphon 20°C water to the 2000 ml line in a 2 liter graduated cylinder.	1a. If you prime the siphon, "waste" about 50 ml of liquid before filling the cylinder.	
		1b. Do not cause splashing which might create air bubbles. Allow the water to run down the sides of the cylinder.	
		1c. Volumes of dilution water larger than 2000 ml may be prepared if needed.	
		1d. If larger amounts of dilution water are needed, Use one ml of each of the four solutions (B.2., B.3., B.4., and B.5.) for each liter of distilled water.	V.B.6.2. (p. 18)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)	2. Add 2.0 ml each of the buffer, magnesium sulfate, calcium chloride, and ferric chloride solutions.	2a. Use a 2 ml volumetric pipet for each solution. 2b. Mix gently with a plunger-type mixer after each solution is added. 2c. The mixture of the four solutions and distilled water is called dilution water.	
C. Procedure 1. Blanks	1. Fill two BOD bottles with dilution water by siphoning.	1a. Rinse the siphon if it's the same one used above. 1b. Hold the siphon about 1/2 inch from the bottom of the bottle before opening the siphon. 1c. Open the siphon slowly. 1d. As the liquid level rises in the bottles, keep the end of the siphon about 1/2 inch above the liquid. 1e. Allow a few ml of liquid to overflow the top of the BOD bottles.	
	2. Stopper the bottles.	2a. Do not cause formation of an air bubble by inserting the stopper too vigorously. 2b. These two bottles are called blanks. 2c. The DO in one bottle from each pair (two bottles will be filled for each sample dilution) and in one blank bottle must be determined within 15 min. of filling, so proceed immediately to C.2., C.3., and C.4. below.	
2. Sample Dilution	1. Thoroughly mix the contents of the sample container. measure the sample, and add it to a 1 liter graduated cylinder.	1a. Use the appropriate size graduated cylinder to measure the sample volume. 1b. Pour the sample down the sides of the graduated cylinder. 1c. During the five-day incubation period, there must be a depletion of at least 2 mg of DO/l, and at least 1 mg of DO/l must remain. 1d. In order to meet these two requirements, it may be necessary to set up two or three dilutions of each sample. Use a separate 1 liter graduated cylinder for each dilution.	V.C.1.1. (p. 18)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Procedure (Continued)		1e. Following, are some suggested sample volumes, although the actual amount of sample to use for each kind of waste may have to be determined on the basis of experience. For effluents of primary treatment plants treating domestic wastewater: 10.0 ml (1% of the liter volume) 20.0 ml (2% of the liter volume) 40.0 ml (4% of the liter volume) For effluents of secondary treatment plants treating domestic wastewaters: 40.0 ml (4% of the liter volume) 60 ml (6% of the liter volume) 80 ml (8% of the liter volume) For effluents from secondary treatment plants: 200 ml (20% of the liter volume) 300 ml (30% of the liter volume) 400 ml (40% of the liter volume)	
		1f. After experience is gained, it will be necessary to use only one dilution for each kind of waste.	
		1g. Measure the sample volumes with an appropriate size graduated cylinder; e.g., 10 ml size for 10 ml of sample, etc.	
	2. Siphon in dilution water to the 1000 ml line.	2a. Rinse the siphon if it's the same one used above. 2b. Use the same technique as in B.6.1.1a. and 1b. above.	
	3. Mix gently with a plunger-type mixer.		
3. BOD bottle filling	1. For each sample dilution set up, fill 2 BOD bottles, by siphoning, from the liter cylinder.	1a. Use the same technique as in C.1.1a. through 1e. above.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Procedure (Continued)			
	2. Stopper the BOD bottles.	2a. Do not cause formation of an air bubble by inserting the stopper too vigorously.	
4. DO Determination	1. Fill the flared top of one of the blank bottles, and one of the two bottles filled for each sample dilution, with water.		
	2. Store them at 20°C in the dark for 5 days.	2a. Check the flared tops daily and refill with water if necessary.	V.C.4.2. (p. 19)
	3. Determine the DO of the 2nd blank bottle and the other sample bottle.	3a. Use the Winkler method-azide modification, or a dissolved oxygen meter. 3b. These DO values are called initial DO values. 3c. Remember the 15 min. mentioned above.	
	4. Record the results.	4a. Page 1-13 is an example data sheet.	
	5. After 5 days, determine the oxygen content of the stored sample and blank BOD bottles.	5a. Use the same method as before.	
	6. Record the results.	6a. Page 1-13 is an example data sheet.	
D. Calculations			
1. Sample	1. For each pair of sample bottles, subtract the DO (in mg/l) after five days from the initial DO (in mg/l).	1a. Example calculation: 7.0 mg/l = initial DO 2.0 mg/l = DO after 5 days $\overline{5.0\ mg/l}$ = DO depletion during the 5 days.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Calculations (Continued)	2. Divide the depletion by the % of sample used (expressed as a decimal) to get the BOD_5 in mg/l.	2a. Example calculation continued: The sample was 40 ml of a primary treatment plant effluent. The 40 ml were diluted to 1000 ml with dilution water. $\frac{40}{1000}$ x 100 = 4% dilution 4% = 0.04 as a decimal mg BOD_5/l = $\frac{5.0}{0.04}$ = 125	
2. Blank	3. For the blank BOD bottles substract the ml of sodium thiosulfate titrant used for the stored bottle from the ml used for the initial bottle.	3a. The difference is used to calculate a "BOD_5" for the blank. 3b. Example calculation: 7.5 mg/l = initial DO 7.3 mg/l = DO after 5 days 0.2 mg/l = DO depletion during the 5 days 3c. The blank may be considered as a 100% (1.0 as a decimal) sample. 3d. mg BOD_5/l for the blank = $\frac{0.2\ mg/l}{1.0}$ = 0.2 3e. If it is greater than 0.2 mg/l, the 20°C water is of low quality. 3f. Possible causes are organic contamination in the water (check the aeration procedure) or dirty glassware (especially the BOD bottles and water storage jug) which has contaminated the water. 3g. This difference is not used as a blank correction, but merely as a check on the quality of the 20°C water.	

Example Data Sheet

Five-Day Biochemical Oxygen Demand (BOD$_5$)

1. Sample number or other identification ______
2. ml of sample used ______
3. % dilution (divide line 2 by 10; assumes dilution to 1,000 ml) ______
4. Decimal equivalent of % dilution (move the decimal point in line 3 two places left) ______
5. Initial sample DO in mg/l ______
6. ml of titrant to titrate initial blank ______
7. Date of initial DO determination ______
8. Time of initial DO determination ______
9. Sample DO after 5 days in mg/l ______
10. ml of titrant to titrate 5th day blank ______
11. Date of 5th day DO determination ______
12. Time of 5th day DO determination ______
13. Line 6 minus line 10* ______
14. Line 5 minus line 9 ______
15. BOD$_5$ in mg/l (line 14 divided by line 4) ______

(ADDITIONAL COLUMNS MAY BE ADDED FOR OTHER SAMPLE DILUTIONS)

*The number here must not be greater than 0.2.

TRAINING GUIDE

SECTION	TOPIC
I	Introduction
II	Educational Concepts - Mathematics
III	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field & Laboratory Equipment
VI	Field & Laboratory Reagents
VII	Field & Laboratory Analysis
VIII	Safety
IX	Records & Reports

*Training guide materials are presented here under the headings marked *.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.1.1	If the glassware is especially dirty and cannot be cleaned with ordinary detergents, chromic acid cleaning may be required.	
	1. Pour 35 ml of distilled water in a 250 ml beaker.	14th Standard Methods p. 336, section 2.c.2)
	2. Add about 1/8 teaspoon (simply estimate this quantity) of sodium dichromate, $Na_2Cr_2O_7$ to the water.	
	3. Swirl the beaker until the sodium dichromate has dissolved.	
	4. Keep repeating steps 2 and 3 until no more sodium dichromate will dissolve.	
	5. Pour the solution into a 2 liter beaker.	
	6. Slowly pour 1 liter of concentrated sulfuric acid, H_2SO_4, into the 2 liter beaker. Caution: Use eyeglasses and protective clothing.	
	7. Stir the mixture thoroughly.	
	8. Store it in a glass-stoppered bottle.	
	9. The cleaning solution should be at a temperature of about 50°C when it is used.	
	10. It may therefore be necessary to warm the cleaning solution.	
	11. When using the warm cleaning solution, fill the piece of glassware with the solution.	
	12. Allow it to soak for 2-3 minutes (or longer).	
	13. Pour the cleaning solution back into the storage bottle.	
	14. Rinse the piece of glassware ten times with tap water.	
	15. The cleaning solution may be reused until it turns green.	
	16. It should then be discarded.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES

14th Standard Methods does not specify a volume tolerance for the BOD bottles. A tolerance of ± 3 ml is suggested by: Methods for Chemical Analysis of Water & Wastes 1974, U.S. Environmental Protection Agency. One method of checking the bottle volume is as follows:

1. Clean the following items as described in section A.1, page 7.

    a. One 250 ml graduated cylinder.
    b. One 100.0 ml volumetric pipet.
    c. One 10.0 ml graduated pipet.
    d. One 500 ml Erlenmeyer flask.

2. Allow the glassware to drain dry.

3. Turn on the hot and cold water taps at the laboratory sink.

4. Adjust the hot and cold water so the temperature of the water is 20°C. Check it with a thermometer.

5. Fill the 500 ml Erlenmeyer flask with the 20°C water.

6. Using the 100 ml volumetric pipet, place 300 ml of the 20°C water in the 250 ml graduated cylinder. (The meniscus will of course be above the 0 graduation line.)

7. Using the 10.0 ml graduated pipet, add 3.0 ml of the 20°C water to the same cylinder.

8. Allow the pipet to drain into the sink and shake it so as to remove water from the tip.

9. Place a mark at the bottom of the meniscus. This is the 303.0 ml graduation mark.

10. Using the same 10.0 ml graduated pipet, remove 10.0 ml of the 20°C water from the 250 ml graduated cylinder.

11. Into the sink drain 6.0 ml of water from the pipet.

12. Very gently blow the rest of the water in the pipet back into the 250 ml graduated cylinder.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
	13. Place a mark at the bottom of the meniscus. This is the 297.0 ml graduation mark. 14. Empty the graduated cylinder and allow it to drain dry. 15. Fill the BOD bottle, whose volume is to be checked, to overflowing with 20°C water. 16. Carefully insert the stopper. There must be no air bubbles in the bottle. 17. Hold one finger over the stopper and invert the bottle so as to drain all water from the flared top. 18. Hold the bottle upright and carefully remove the stopper. 19. Carefully pour the entire contents of the bottle into the 250 ml graduated cylinder. 20. If the meniscus is between the 297.0 and 303.0 ml graduation marks, the BOD bottle may be used. If the meniscus is not, the bottle should not be used for the BOD_5 test.	

B.6.2. If just enough dilution water is prepared for use at one time, then the procedure in B.6. would be followed. If you desire to keep a supply of dilution water, add 1 ml of the magnesium, calcium, and ferric solutions for each 1 liter of distilled water. But do not add the buffer until you are ready to use the dilution water. The water should be stored in the dark at 20°C ± 1°C.

C.1.1. Standard Methods, 14th ed., cites two other ways of diluting the sample.

1. Directly in the 300 ml BOD bottle

   Example calculation: A 4% dilution of the sample is to be made.

   300 ml = volume of BOD bottle
   .04 = % dilution expressed as a decimal
   12.00 = ml of sample to be diluted in the BOD bottle

   Measure the 12 ml of sample, add it directly to the BOD bottle, and fill the bottle to overflowing with dilution water.

2. If the dilution will be less than 1%, it should be done in a volumetric flask.

   Example calculation: A 0.5% dilution of the sample is to be made. Since two BOD bottles must be filled, make the dilution in a 1 liter volumetric flask.

   1000 ml = volume of flask
   .005 = %dilution expressed as a decimal
   5.000 ml = ml of sample to be diluted in the flask

   Measure the 5 ml of sample, add it directly to the flask, and fill to the mark with dilution water. Fill the two BOD bottles to overflowing by siphoning from the flask.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
C.4.2.	There are other ways of keeping the water seal on the BOD bottle; e.g., the bottles may be turned upside down in a BOD pan (rectangular pan with square compartments) half full of tap water or plastic caps (made for BOD bottles) may be put over the bottle tops.	

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

WINKLER DETERMINATION OF DISSOLVED OXYGEN-AZIDE MODIFICATION

as applied in

WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

Developed by the

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

EFFLUENT MONITORING PROCEDURE: Winkler Determination of Dissolved Oxygen-Azide Modification

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EFFLUENT MONITORING PROCEDURE: Winkler Determination of Dissolved Oxygen-Azide Modification

1. Analysis Objectives:

The operator will be able to perform a Winkler dissolved oxygen determination, using the azide modification, on a sewage sample.

2. Brief Description of Analysis:

A solution of manganous sulfate is added to the sample. A solution containing sodium hydroxide, sodium iodide and sodium azide is next added. If oxygen is present in the sample, a brown flocculent precipitate forms. If no oxygen is present, a white precipitate forms. Sulfuric acid is then added to the sample, and the precipitate dissolves. The solution is titrated with sodium thiosulfate using starch indicator. At the end point of the titration, the color of the solution changes from pale blue to colorless. The milliliters of sodium thiosulfate used, is equal to the milligrams of dissolved oxygen per liter of sample.

3. Applicability of this Procedure:

This effluent monitoring procedure describes the determination of dissolved oxygen. The analysis is performed as part of the five-day biochemical oxygen demand test. Interferences/sample pretreatment are therefore mentioned in the Effluent Monitoring Procedure, Determination of Five-Day Biochemical Oxygen Demand (BOD_5).

This procedure was excerpted from Methods for Chemical Analysis of Water and Wastes 1974, Methods Development and Quality Assurance Research Laboratory, National Environmental Research Center, Cincinnati, Ohio 45268.

EFFLUENT MONITORING PROCEDURE: Winkler Determination of Dissolved Oxygen-Azide Modification

General Description of Equipment Used in the Process

A. Capital Equipment

1. Analytical balance, 200 g. capacity
2. Trip balance, 500 g. capacity
3. Oven, temperature controlable to ± 2°C, large enough to hold a small evaporating dish
4. Refrigerator, large enough to hold three 1 liter bottles
5. Still, or other source of distilled water

B. Reusable

1. Hot plate, large enough to hold a 2 liter Erlenmeyer flask
2. Kemmerer sampler
3. APHA sampler
4. Laboratory apron
5. Safety glasses
6. Brushes (for cleaning glassware)
7. Brush (for cleaning balance)
8. One 1 liter volumetric flask
9. One 300 ml (± 3 ml) BOD bottle (see page 18)
10. One 1 liter graduated cylinder
11. One 100 ml graduated cylinder
12. One 50 ml graduated cylinder
13. One 10 ml graduated cylinder
14. Six 1 liter glass stoppered bottles
15. One rubber stopper (to fit a 1 liter glass stoppered bottle)
16. One 150 ml glass stoppered bottle
17. One spatula (medium size)
18. One spatula (small size)
19. One 2 liter Erlenmeyer flask
20. One 500 ml wide mouth Erlenmeyer flask
21. One 100 ml pipet
22. One 50 ml pipet
23. One 20 ml pipet
24. One pipet bulb
25. Three 5 ml graduated pipets
26. One desiccator (large enough to hold a small evaporating dish)
27. One evaporating dish (large enough to hold about 10 g. of solid)
28. One 25 ml buret
29. One ring stand
30. One buret clamp
31. One distilled water plastic squeeze bottle
32. One pen or pencil
33. One notebook (for recording data)
34. Eight plastic weighing boats (2-3 inches square)

B. Reusable Supplies (Continued)

35. Sponges (for cleaning of laboratory table tops)
36. One stirring rod (about 6 inches long)
37. One powder funnel, about 3 inch diameter

C. Consumable Supplies:

1. Potassium dichromate, $K_2Cr_2O_7$
2. Concentrated sulfuric acid, H_2SO_4
3. Soap
   (These three reagents are for cleaning glassware. The quantitites needed will therefore vary.)
4. 480 g. manganous sulfate tetrahydrate, $MnSO_4 \cdot 4H_2O$
   400 g. manganous sulfate dihydrate, $MnSO_4 \cdot 2H_2O$, or 364 g. manganous sulfate monohydrate, $MnSO_4 \cdot H_2O$ may also be used.
5. 500 g. sodium hydroxide, NaOH
6. 135 g. sodium iodide, NaI
7. 10 g. sodium azide, NaN_3
8. 10 g. soluble starch
9. 15 ml chloroform, $CHCl_3$
10. 186.15 g. sodium thiosulfate pentahydrate, $Na_2S_2O_3 \cdot 5H_2O$
11. 6 g. potassium biiodate, $KH(IO_3)_2$
12. 3 g. potassium iodide, KI
13. 10 ml concentrated sulfuric acid, H_2SO_4
14. Sodium dichromate, $Na_2Cr_2O_7$

The quantities given in 4 through 11 above will suffice for approximately 450 determinations of dissolved oxygen. Depending on usage, smaller quantities may be prepared.

All reagents should be of high quality. Different chemical manufacturers may have different ways of indicating a high quality reagent. While no endorsement of one chemical manufacturer over another is intended, the following are some designations used in four chemical catalogs to indicate high quality reagents.

Catalog	Designations
Thomas	Reagent, ACS, Chemically Pure (CP)
Matheson, Coleman & Bell	Reagent, ACS
Curtin Matheson Scientific, Inc.	Primary Standard, ACS, AR
Fisher	Certified, ACS

EFFLUENT MONITORING PROCEDURE: Winkler Determination of Dissolved Oxygen-Azide Modification

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Equipment Preparation 1. Cleaning of glassware	1. Clean all glassware and rinse with distilled water.		V.A.1.1 (p. 17)
2. Balance preparation	1. Check all balances for cleanliness and proper operation.		
B. Reagent Preparation 1. Manganous sulfate solution	1. Prepare 1 liter of solution containing 480 g. of manganous sulfate tetratrydrate, $MnSO_4 \cdot 4H_2O$	1a. Unless otherwise specified, solutions should be stored in glass stoppered bottles. 1b. Unless otherwise specified, the term water means distilled water.	
2. Alkaline iodide azide solution	1. Dissolve 500 g. of sodium hydroxide, NaOH, in 500 ml of water.	1a. Caution: heat is generated	
	2. Cool the solution to room temperature.		
	3. Dissolve 135 g. of sodium iodide, NaI, in 200 ml of water.		
	4. Dissolve 10 g. of sodium azide, NaN_3, in 40 ml of water.		
	5. Combine the three solutions and dilute to 1 liter.	5a. This solution should be stored in a glass bottle fitted with a rubber stopper, or in a clean plastic bottle.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
3. Starch solution	1. Gently boil 1 liter of water on a hot plate.	1a. Proceed with the next steps while the water is heating and boiling.	
	2. Weigh 10 g. of soluble starch.		
	3. Transfer it to a mortar.		
	4. Add about 3 ml of water.		
	5. Grind with a pestle so as to form a thin paste.		
	6. Pour the paste into the boiling water.		
	7. Allow the solution to stand overnight.		
	8. Decant the starch solution into a bottle.	8a. Decanting means to pour slowly so that any solid material will be left behind.	
	9. Add 5 ml of chloroform, $CHCl_3$.	9a. Store in a refrigerator.	
4. Sodium Thiosulfate stock solution 0.75 N (approximate)	1. Boil 1500 ml of water for 3 minutes		
	2. Cool the water to room temperature.		
	3. Weigh 186.15 g. of sodium thiosulfate pentahydrate, $Na_2S_2O_3 \cdot 5H_2O$.		

EFFLUENT MONITORING PROCEDURE: Winkler Determination of Dissolved Oxygen-Azide Modification

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
4. Continued	4. Dissolve in 500 ml of the water.		
	5. Dilute to 1 liter with more of the water.		
	6. Add 5 ml of chloroform, $CHCl_3$.	6a. Store in a refrigerator.	
5. Sodium thiosulfate standard titrant, 0.0375N (approximate)	1. Dilute 50.0 ml of the sodium thiosulfate stock solution to 1 liter.	1a. Do not transfer any of the chloroform from the stock solution.	
	2. Add 5 ml of chloroform, $CHCl_3$.	2a. Store in a refrigerator.	
6. Potassium biiodate standard, 0.0375 N	1. Dry 6 g. of potassium biiodate, $KH(IO_3)_2$ at 103°C for 2 hours.		
	2. Cool in a desiccator.		
	3. Prepare 1 liter of a solution containing 4.837 g. of the potassium biiodate.		
	4. Dilute 250.0 ml of this solution to 1 liter.	4a. The N of this solution is 0.0375.	
7. Sulfuric acid, 10% by volume	1. Pour 10 ml of concentrated sulfuric acid, H_2SO_4, into 90 ml of water	1a. Caution: pour the acid slowly. Mix after each addition of 2 ml of the acid.	
	2. Cool the solution to room temperature.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Standardization of Sodium Thiosulfate Standard Titrant			
1. Potassium biiodate standard, 0.0375 N	1. Weigh 1-3 g. of potassium iodide, KI.		
	2. Dissolve in 100-150 ml of water.		
	3. Add 10 ml of 10% by volume sulfuric acid.		
	4. Add 20.0 ml of the 0.0375 N potassium biiodate.	4a. Use a volumetric pipette.	
	5. Place the solution in the dark for 5 minutes.		
	6. Add water so as to bring the volume to 300 ml.		
2. Titration	1. Add the approximately 0.0375 N sodium thiosulfate titrant to the solution from a buret until the color changes from red-brown to pale yellow.		
	2. Add 2 ml of starch solution.	2a. A medium blue-pale blue color will form.	
	3. Continue the titration until the color changes from pale blue to colorless.	3a. Ignore any return of blue color.	
	4. Record the ml of sodium thiosulfate used.		

EFFLUENT MONITORING PROCEDURE: Winkler Determination of Dissolved Oxygen-Azide Modification

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
3. Calculations	1. Divide the ml of sodium thiosulfate used into 0.75.	1a. The result is the normality of the sodium thiosulfate titrant. It is desirable, but not necessary, that the normality be exactly 0.0375.	II.C.3.1 (p. 16)
D. Determination of Dissolved Oxygen			
1. Sample collection	1. If the sample is to be collected from a depth greater than 5 feet, use a Kemmerer sampler.		
	2. If the sample is to be collected from a depth less than 5 feet use an APHA sampler containing a 300 ml BOD bottle.		
	3. If a Kemmerer is used, transfer the sample to a 300 ml BOD bottle. Allow some of the sample to overflow.	3a. Caution: during the sample transfer, do not allow it to splash.	
	4. Carefully insert the stopper of the BOD bottle.	4a. Do not create any air bubbles in the bottle.	
	5. For surface samples, the sample may be collected directly in a 300 ml BOD bottle.	5a. Fill the bottle in such a way that no turbulence is created.	
2. Addition of reagents	1. Remove the stopper and pipette 2.0 ml of manganous sulfate solution into the sample.	1a. Have the tip of the pipette about 1/2 inch below the surface of the liquid. It is desirable, but not necessary, that the normality be 0.0375.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
2. Continued	2. Pipette 2.0 ml of alkaline iodide azide solution into the sample, over the sink.	2a. Have the tip of the pipette about 1/2 inch below the surface of the liquid. 2b. A precipitate forms.	
	3. Carefully insert the stopper of the BOD bottle.	3a. Do not create any air bubbles in the bottle.	
	4. Rinse off the outside of the BOD bottle.	4a. The alkaline iodide azide solution is damaging to the skin.	
	5. Holding the hand over the stopper, invert the BOD bottle slowly 5 times.		
	6. Allow the precipitate to settle.	6a. If it does not settle, wait 2 minutes and proceed.	
	7. Repeat the shaking and settling steps.		
	8. Pipette 2.0 ml of concentrated sulfuric acid into the sample.	8a. The pipette need not be below the surface of the liquid.	
	9. Carefully insert the stopper of the BOD bottle.	9a. Do not create any air bubbles in bottle during this step.	
	10. Rinse off the outside of the BOD bottle.		
	11. Holding the hand over the stopper, invert the BOD bottle slowly five times.	11a. The precipitate will dissolve. 11b. The color of the solution is red-brown if oxygen is present, but colorless if no oxygen is present. If the solution is yellow, a small amount of oxygen is present.	

EFFLUENT MONITORING PROCEDURE: Winkler Determination of Dissolved Oxygen-Azide Modification

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
3. Titration	1. Transfer the entire contents of the 300 ml BOD bottle to a wide mouth 500 ml Erlenmeyer flask.		
	2. Add the sodium thiosulfate titrant from a buret until the red-brown color changes to a pale yellow.	2a. If there was little oxygen in the sample and the yellow color was therefore present even before addition of any sodium thiosulfate, the starch should be added immediately.	
	3. Add 2 ml of starch solution.	3a. A medium blue-pale blue color will form.	
	4. Continue the titration until the color changes from pale blue to colorless.	4a. Ignore any return of blue color.	
	5. Record the ml of sodium thiosulfate titrant used.		
4. Calculations	1. Calculate the mg of DO per liter of sample.	1a. mg DO/liter = ml of sodium thiosulfate titrant X N of sodium thiosulfate titrant X 8 x 1000/ml of sample 1b. Since the sample was in a 300 ml BOD bottle, mg DO/liter = ml of sodium thiosulfate X N of sodium thiosulfate X 8 x 1000/300 1c. or, mg DO/liter = ml of sodium thiosulfate X N of sodium thiosulfate x 26.7	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
4. Continued		1d. If the N of the sodium thiosulfate was exactly 0.0375, then mg DO/liter = ml of sodium thiosulfate x 0.0375 x 8 x 1000/300 1e. or, mg DO/liter = ml of sodium thiosulfate x 1	

TRAINING GUIDE

SECTION	TOPIC
I	Introduction
II*	Educational Concepts - Mathematics
III	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field & Laboratory Equipment
VI	Field & Laboratory Reagents
VII	Field & Laboratory Analysis
VIII	Safety
IX	Records & Reports

*Training guide materials are presented here under the headings marked *.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
C.3.1	The formula used is: normality of sodium thiosulfate x ml of sodium thiosulfate = normality of potassium biiodate x ml of potassium biiodate. Three of the four values are known: ml of sodium thiosulfate is read from the buret. ml of potassium biiodate = 20.0 normality of potassium biiodate = 0.0375 After rearranging the formula to solve for the normality of sodium thiosulfate, and inserting the known values: Normality of sodium thiosulfate = 20.0 x 0.0375/ml of sodium thiosulfate = 0.75/ml of sodium thiosulfate	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.1.1	If the glassware is especially dirty and cannot be cleaned with ordinary detergents, chromic acid cleaning may be required.	
	1. Pour 35 ml of distilled water in a 250 ml beaker.	14th Standard Methods, p. 336, section 2.c.2)
	2. Add about 1/8 teaspoon (simply estimate this quantity) of sodium dichromate $Na_2Cr_2O_7$ to the water.	
	3. Swirl the beaker until the sodium dichromate has dissolved.	
	4. Keep repeating steps 2 and 3 until no more sodium dichromate will dissolve.	
	5. Pour the solution into a 2 liter beaker.	
	6. Slowly pour 1 liter of concentrated sulfuric acid, H_2SO_4, into the 2 liter beaker. Caution: Use eyeglasses and protective clothing.	
	7. Stir the mixture throughly.	
	8. Store it in a glass stoppered bottle.	
	9. The cleaning solution should be at a temperature of about 50°C when it is used.	
	10. It may therefore be necessary to warm the cleaning solution.	
	11. When using the warm cleaning solution, fill the piece of glassware with the solution.	
	12. Allow it to soak for 2-3 minutes (or longer).	
	13. Pour the cleaning solution back into the storage bottle.	
	14. Rinse the piece of glassware ten times with tap water.	
	15. The cleaning solution may be reused until it turns green.	
	16. It should then be discarded.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES

13th Standard Methods does not specify a volume tolerance for the BOD bottles. A tolerance of ± 3 ml is suggested by: Methods for Chemical Analysis of Water & Wastes 1974, U.S. Environmental Protection Agency. One method of checking the bottle volume is as follows:

1. Clean the following items as described in section A.1, page 7.

    a. One 250 ml graduated cylinder.
    b. One 100.0 ml volumetric pipet.
    c. One 10.0 ml graduated pipet.
    d. One 500 ml Erlenmeyer flask.

2. Allow the glassware to drain dry.

3. Turn on the hot and cold water taps at the laboratory sink.

4. Adjust the hot and cold water so the temperature of the water is 20°C. Check it with a thermometer.

5. Fill the 500 ml Erlenmeyer flask with the 20°C water.

6. Using the 100 ml volumetric pipet, place 300 ml of the 20°C water in the 250 ml graduated cylinder. (The meniscus will of course be above the 0 graduation line.)

7. Using the 10.0 ml graduated pipet, add 3.0 ml of the 20°C water to the same cylinder.

8. Allow the pipet to drain into the sink and shake it so as to remove water from the tip.

9. Place a mark at the bottom of the meniscus. This is the 303.0 ml graduation mark.

10. Using the same 10.0 ml graduated pipet, remove 10.0 ml of the 20°C water from the 250 ml graduated cylinder.

11. Into the sink drain 6.0 ml of water from the pipet.

12. Very gently blow the rest of the water in the pipet back into the 250 ml graduated cylinder.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
	13. Place a mark at the bottom of the meniscus. This is the 297.0 ml graduation mark. 14. Empty the graduated cylinder and allow it to drain dry. 15. Fill the BOD bottle, whose volume is to be checked, to overflowing with 20°C water. 16. Carefully insert the stopper. There must be no air bubbles in the bottle. 17. Hold one finger over the stopper and invert the bottle so as to drain all water from the flared top. 18. Hold the bottle upright and carefully remove the stopper. 19. Carefully pour the entire contents of the bottle into the 250 ml graduated cylinder. 20. If the meniscus is between the 297.0 and 303.0 ml graduation marks, the BOD bottle may be used. If the meniscus is not, the bottle should not be used for the BOD_5 test.	

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

DECHLORINATION OF SAMPLES FOR BIOCHEMICAL OXYGEN DEMAND AND SEEDING OF THE DILUTION WATER

as applied in

WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

Developed by the

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

EFFLUENT MONITORING PROCEDURE: Dechlorination of Samples for Biochemical Oxygen Demand and Seeding of the Dilution Water

This operational procedure was developed by:

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POSITION Chemist-Instructor

EDUCATION AND TECHNICAL BACKGROUND

B.S. - Chemistry

M.S. - Chemistry

1-1/2 years Industrial Chemist

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EFFLUENT MONITORING PROCEDURE: Dechlorination of Samples for Biochemical Oxygen Demand and Seeding of the Dilution Water

1. Analysis Objectives:

The learner will dechlorinate a sample of wastewater treatment plant effluent, and seed a supply of dilution water for use in the biochemical oxygen demand test.

2. Brief Description of Analysis:

Chlorine is added to wastewater treatment plant effluents in order to destroy undesirable bacteria. Part of the chlorine is used up by chemical reaction with pollutants in the effluent. The resulting chlorine compounds, and the chlorine which has destroyed the bacteria, are an interference in the biochemical oxygen demand test. If the chlorine is not chemically "neutralized," the biochemical oxygen demand results will be meaningless. The chemical "neutralization" is accomplished by adding a calculated amount of sodium sulfite solution to the sample.

Because bacteria in the sample have been destroyed by the chlorination step, they must be replaced in order to carry out the biochemical oxygen demand test. This is accomplished by introducing bacteria from domestic sewage into the water used to dilute the dechlorinated sample.

3. Applicability of this Procedure:

   a. Theoretically, this procedure can be used to "neutralize" any concentration of chlorine. In practice, the concentration which would have to be "neutralized" would probably not exceed 2 or 3 mg/liter.

   b. Any needed preservation techniques would be those required for the biochemical oxygen demand test itself. Any loss of chlorine, such as through agitation, or exposure to sunlight, would actually be beneficial, since there would be less to "neutralize."

   c. If the biochemical oxygen demand sample is taken prior to chlorination, the "neutralization" and seeding steps described in this procedure are unnecessary. In this case, the effluent monitoring procedure, Determination of Five-Day Biochemical Oxygen Demand (BOD_5), should be used.

Source of Procedure: Standard Methods, 14th ed., par. 4.c.2) page 546

EFFLUENT MONITORING PROCEDURE: Dechlorination of Samples for Biochemical Oxygen Demand and Seeding of the Dilution Water

Dechlorinate 100 ml of sample

↓

Calculate amount of sodium sulfite to dechlorinate entire sample

↓

Dechlorinate entire sample

↓

Check completeness of dechlorination

↓

Prepare seed controls

↓

Seed the dilution water

↓

Do initial and final dissolved oxygen determinations on the seed controls and the dechlorinated sample

↓

Final calculations

EFFLUENT MONITORING PROCEDURE: Dechlorination of Samples for Biochemical Oxygen Demand and Seeding of the Dilution Water

General Description of Equipment Used in the Process

A. Capital Equipment:

1. Trip balance, 100 g capacity
2. Analytical balance
3. Still, or other source of distilled water
4. One incubator, 20°C ± 1°C (large enough for 6 BOD bottles and a 2 liter Erlenmeyer flask

B. Reusable Supplies:

1. Brushes (for cleaning glassware)
2. Brush (for cleaning balance)
3. Laboratory apron
4. Safety glasses
5. One distilled water plastic squeeze bottle
6. One pen or pencil
7. One notebook (for recording data)
8. Sponge (for cleaning laboratory table top)
9. Three 1 liter graduated cylinders
10. One 500 ml graduated cylinder
11. One 250 ml graduated cylinder
12. One 100 ml graduated cylinder
13. One 50 ml graduated cylinder
14. One 10 ml graduated cylinder
15. One 250 ml beaker
16. One hot plate
17. One magnetic stirrer (optional)
18. One magnetic stirring bar (optional; about 1 inch long)
19. One small spatula (for use when weighing solids)
20. One 25 ml buret
21. One small funnel (to fit in the top of the buret)
22. One clamp (to support the buret)
23. One ring stand (for use with the buret and clamp)
24. One mortar and pestle (about 100 ml capacity)
25. One eyedropper
26. One 1 ft. long stirring rod
27. One 10 ml graduated pipet
28. One 100 ml volumetric pipet
29. One 50 ml volumetric pipet
30. Two 1 liter glass-stoppered bottles
31. Two 100 ml glass-stoppered bottles
32. Two 2 liter Erlenmeyer flasks
33. One 500 ml Erlenmeyer flask
34. One 250 ml Erlenmeyer flask
35. Four plastic weighing boats (2 inches on an edge)
36. One Erlenmeyer flask or large bottle containing about 5 liters of dilution water at 20°C ± 1°C. (See the effluent monitoring procedure, Determination of Five-Day Biochemical Oxygen Demand, BOD_5, sections B.1. through B.6. for its preparation.

B. Reusable Supplies (Continued)

37. One siphon (long enough to reach to the bottom of the above container)
38. One siphon (long enough to reach to the bottom of a 1 liter graduated cylinder, item 37 may be used if it is thoroughly rinsed)
39. One plunger type mixer (for use with the 1 liter graduated cylinders)
40. Twelve 300 ml (± 3 ml) BOD bottles
41. One pipet bulb
42. One 1 liter volumetric flask
43. Asbestos gloves or crucible tongs
44. Equipment for determination of dissolved oxygen by the Winkler Method-azide modification, or by the use of a dissolved oxygen meter. See the appropriate effluent monitoring procedure.

C. Consumable Supplies:

1. Concentrated sulfuric acid, H_2SO_4, 5 ml
2. Potassium iodide, KI, 10 g
3. Anhydrous sodium sulfite, Na_2SO_3, 2 g
4. Soluble starch, 5 g
5. Salicylic acid, $C_7H_6O_3$, 1.25 g
6. Reagents for determination of dissolved oxygen by the Winkler Method, azide modification, or by the use of a dissolved oxygen meter.

All reagents should be of high quality. Different chemical manufacturers may have different ways of indicating a high quality reagent. While no endorsement of one chemical manufacturer over another is intended, the following are some designations used in four chemical catalogs to indicate high quality reagents.

Catalog	Designations
Thomas	Reagent, ACS, Chemically Pure (CP)
Matheson, Coleman & Bell	Reagent, ACS
Curtin Matheson Scientific, Inc.	Primary Standard, ACS, AR
Fisher	Certified, ACS

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Equipment Preparation			
1. Cleaning of glassware	1. Clean all glassware and rinse with distilled water.	1a. Throughout the remainder of this procedure, unless otherwise stated, the word water means distilled water.	V.A.1.1
2. Balance inspection	1. Check all balances for cleanliness and proper operation.	1a. Consult the manual supplied with the balance if you are unable to correct any malfunction of the balance.	
B. Reagent Preparation			
1. Seed material	1. Collect 1 liter of domestic sewage influent in a 2 liter Erlenmeyer flask.	1a. Simply estimate this volume.	
	2. Place the flask in an incubator for 24-36 hours.	2a. At $20^{\circ}C \pm 1^{\circ}C$. 2b. There should be no stopper in, or cover on, the flask. 2c. During normal working hours, swirl the flask for about 1 minute every 2 hours. This will ensure that the sewage is thoroughly mixed with oxygen from the air. 2d. The solids in the sewage must be completely settled when the sewage is used in this procedure. Therefore, the last swirling should be done at least 2 hours before use. 2e. These steps must be done 24-36 hours before the sample is to be dechlorinated and the BOD set up, in order to prevent any delay in the procedure. 2f. The supernatant liquid above the solid material is called seed. 2g. This seed material will be used in sections F.1. and F.3.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)			
2. Sulfuric acid solution, H_2SO_4, 1 + 50	1. Measure 50 ml of distilled water.	1a. Use a 50 ml graduated cylinder.	
	2. Pour it into a 250 ml Erlenmeyer flask.		
	3. Measure 1 ml of concentrated sulfuric acid, H_2SO_4.	3a. Use a 10 ml graduated cylinder. 3b. It will be more convenient, and safer, to pour 2 or 3 ml of the acid into a small beaker, and pour it from the beaker into the cylinder. The excess acid may be discarded.	
	4. Pour it into the Erlenmeyer flask.		
	5. Swirl the flask to mix the contents.		
	6. Store the solution in a 100 ml glass stoppered bottle.		
3. Potassium iodide solution, KI, 10%	1. Weigh 10 g of potassium iodide, KI.	1a. Use a trip balance. 1b. Use a plastic weighing boat.	
	2. Transfer it to a graduated 250 ml Erlenmeyer flask.		
	3. Add water to the flask to the 100 ml mark.		
	4. Swirl the flask to dissolve the solid.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (Continued)			
	5. Store the solution in a 100 ml glass stoppered bottle.		
4. Sodium sulfite solution, Na_2SO_3, 0.025 N	1. Weigh 1.575 g of anhydrous sodium sulfite, Na_2SO_3.	1a. Use an analytical balance. 1b. Use a plastic weighing boat.	
	2. Transfer it to a 1 liter volumetric flask.		
	3. Fill the flask about half full with water.		
	4. Swirl the flask to dissolve the solid.		
	5. Add water to the 1 liter mark.		
	6. Thoroughly mix the contents of the flask.		
	7. Store the solution in a 1 liter glass stoppered bottle.	7a. The concentration of this solution is 0.025 N. 7b. It is not stable, and must be prepared fresh on each day it is used.	
5. Starch indicator	1. Weigh 5 g of soluble starch.	1a. Use a trip balance. 1b. Use a plastic weighing boat.	
	2. Transfer it to a mortar.		
	3. Measure 1 liter of water.	3a. Use a 1 liter graduated cylinder.	
	4. Pour the water into a 2 liter Erlenmeyer flask.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)			
	5. Bring the water to a boil.	5a. Use a hot plate. 5b. While the water is coming to a boil, do steps 6 and 7.	
	6. While the water is coming to a boil, add 1 ml of water to the starch in the mortar.	6a. Use a 10 ml graduated cylinder.	
	7. Grind the starch and water together.	7a. Use a pestle. 7b. The objective is to form a thin paste. 7c. A few additional drops of water may have to be added.	
	8. Pour the thin paste slowly into the boiling water.		
	9. Invert a 250 ml beaker and place it on top of the Erlenmeyer flask.		
	10. Turn the hot plate off.		
	11. Remove the flask from the hot plate.	11a. Caution: The flask is hot. 11b. Use asbestos gloves or crucible tongs to move the flask.	
	12. Allow the starch solution to stand overnight.		
	13. Carefully decant the supernatant liquid into a 1 liter glass-stoppered bottle.	13a. Recall that decant means to carefully pour out the liquid and leave any solid material behind.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)	14. Weigh 1.25 g of salicylic acid.	14a. Use an analytical balance (or trip balance if it weighs to the second decimal place). 14b. Use a plastic weighing boat.	
	15. Add it to the starch solution in the bottle.		
	16. Swirl the bottle to dissolve the solid.		
6. Dilution water	1. Prepare the needed quantity of dilution water.	1a. See the effluent monitoring procedure on the Determination of Five-Day Biochemical Oxygen Demand, sections B.1. through B.6., for the preparation of the dilution water. Throughout the remainder of this procedure, the EMP on BOD$_5$ will be used to mean the effluent monitoring procedure on the Determination of Five-Day Biochemical Oxygen Demand. 1b. A maximum of 300 ml is needed for each BOD bottle, whether it contains a sample, seed material, or a dilution water blank. 1c. Multiply the number of bottles to be set up by 300 to get the total volume of dilution water needed. Prepare an extra 500 ml for "safety". Six bottles are needed for the seed material, and 2 are needed for the dilution water blank. See the EMP on BOD$_5$, sections C.1. and C.2., for the number of sample bottles needed. 1d. Recall from the EMP on BOD$_5$ that the dilution water should be at $20^\circ \pm 1^\circ$C when used. When not being used, it is stored at this temperature.	

EFFLUENT MONITORING PROCEDURE: Dechlorination of Samples for Biochemical Oxygen Demand and Seeding of the Dilution Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Determination of amount of Sodium Sulfite needed for dechlorination	1. Pipet 100 ml of well mixed sample into a 500 ml Erlenmeyer flask.	1a. Use a volumetric pipet. If solids clog the tip of the pipet, use a graduated cylinder. 1b. See Sections C.1 and C.2. of the EMP on BOD_5 to determine the amount of sample needed. Since 100 ml of sample are needed to determine the quantity of sodium sulfite required for dechlorination, the sample volume collected should be about 100 ml, plus the amount of sample needed for the BOD_5, plus about 200 ml for "safety." Simply estimate the total volume when collecting the sample.	
	2. Measure 10 ml of the sulfuric acid solution.	2a. Use a 10 ml graduated cylinder.	
	3. Add it to the Erlenmeyer flask.		
	4. Swirl the flask to mix the contents.		
	5. Measure 10 ml of the potassium iodide solution.	5a. Use a 10 ml graduated cylinder.	
	6. Add it to the Erlenmeyer flask.		
	7. Swirl the flask to mix the contents.	7a. The color of the solution is red brown.	
	8. Fill a 25 ml buret to the 0.0 line with the 0.025 N sodium sulfite.		
	9. While swirling the flask vigorously, add the sodium sulfite from the buret.	9a. Add the sodium sulfite at a fast, drop-wise rate. 9b. A magnetic stirrer may be used.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Determination of Amount of Sodium Sulfite needed for dechlorination (continued)			
	10. When the red-brown color changes to a pale yellow color, stop the addition of sodium sulfite.		
	11. Measure 2 ml of the starch indicator.	11a. Use a 10 ml graduated cylinder. 11b. Although the cited reference does not specify what volume of starch indicator is to be used, two ml is a commonly used quantity.	
	12. Add it to the flask.		
	13. Swirl the flask to mix the contents.	13a. The solution will be medium or pale blue in color.	
	14. While swirling the flask (or using the magnetic stirrer), begin again to add the sodium sulfite solution from the buret.	14a. At a rate of about 1 drop per second. 14b. The solution will become lighter blue in color. 14c. Read step 15 before carrying out step 14.	
	15. When the solution turns from pale blue to colorless, immediately stop the addition of sodium sulfite.		
	16. Record the ml of sodium sulfite used to one place to the right of the decimal point.	16a. Note that the 100 ml of sample you have just titrated should now be discarded. The actual BOD test will be done on another, and larger portion of sample.	

EFFLUENT MONITORING PROCEDURE: Dechlorination of Samples for Biochemical Oxygen Demand and Seeding of the Dilution Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Calculations	1. Calculate the amount of the 0.025 N sodium sulfite needed to dechlorinate the rest of the biochemical oxygen demand (BOD) sample.	1a. Example calculation: Assume 3.2 ml of the 0.025 N sodium sulfite were used from the buret to titrate the 100 ml of sample; i.e., 3.2 ml of the sodium sulfite were needed to dechlorinate 100 ml. of sample. 1b. Assume 1 liter is the total volume of BOD sample. 1c. ml of 0.025 N sodium sulfite to dechlorinate the rest of the BOD sample = $\frac{\text{ml of entire BOD sample (1000)}}{\text{ml of BOD sample taken for the above test (100)}} \times$ ml of 0.025 N sodium sulfite used from the buret in C. (3.2) $= \frac{1000}{100} \times 3.2$ $= 32$	
	2. Measure the amount of 0.025 N sodium sulfite just calculated.	2a. It was 32 ml in the example calculation. 2b. Use a graduated pipet if the required amount of sodium sulfite is less than 10 ml. If it is greater than 10 ml, use a graduated cylinder.	
	3. Add it to the entire BOD sample.		
	4. Stir or shake the sample container so as to mix the contents.		
	5. Let the container stand for 20 minutes.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Check on Dechlorination	1. Repeat steps C.1 through C.7 above.	1a. However, the color should not be red-brown at this time. It will either be colorless, or pale blue. 1b. Except use 100 ml of BOD sample to which the 0.025 N sodium sulfite solution has already been added. (D.5.)	
	2. Add 2 ml of starch indicator.	2a. Use a 10 ml graduated cylinder.	
	3. Swirl the flask to mix the contents.	3a. If the solution is colorless, it indicates that all of the chlorine has been "neutralized," and the dechlorinated sample may now be used for the BOD test. See the EMP on BOD_5, Sections C.1. and C.2. 3b. If there is any blue color, it indicates that not all of the chlorine interferences have been "neutralized." 3c. If there is blue color, add 2 drops of the 0.025 N sodium sulfite to the BOD sample (1 liter in the example calculation) and mix. 3d. Repeat 3c., E.1, E.2., and E.3., above until the solution is colorless. 3e. When the colorless condition has been achieved, the sample is dechlorinated and may be used for the BOD test. See the Emp on BOD_5, Sections C.1. and C.2.	
	4. Discard the 100 ml of sample on which the check was performed.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Calculation of Oxygen Depletion in the Sample, Due to Seed			
1. Preparation of Seed Controls	1. Pipet 100 ml of the seed (supernatant liquid) used in B.1.2. into a 1 liter graduated cylinder.	1a. In order to calculate the BOD_5 for a sample which has been diluted with seeded dilution water, the oxygen depletion in the sample due to the seed, must be considered. In order to be useful for the final calculation of BOD_5, the five-day oxygen depletion in the seed control (F.1.) must be 40-70%. In F.1. below, three seed dilutions are set up; 10%, 15%, and 20%. At least one of them should give a depletion in the 40-70% range. If more than one dilution gives a depletion in the 40-70% range, use the higher % value for calculation of the BOD_5. The 10, 15, and 20% dilutions may not be high enough to give a 40-70% depletion with some seed material. If not, experience will have to be used. For example, 20, 25, and 30% may have to be used. Three dilutions should still be set up, however. The first step in determining the oxygen depletion due to the seed is to prepare seed controls. The second step is to determine the oxygen depletion in the seed controls. After this, a seed control correction is calculated and applied to the BOD_5 of the sample. Example calculations are used throughout the remainder of this procedure. 1b. Use a 100 ml volumetric pipet.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Calculation of Oxygen Depletion in the Sample, Due to Seed (continued)			
	2. Siphon dilution water (B.6.) ($20^\circ C \pm 1^\circ C$) into the graduated cylinder to the 1000 ml line.	2a. If the siphon was "primed" with water, waste about 50 ml before filling the cylinder. 2b. Cause no splashing of the liquid. 2c. Let the dilution water run down the sides of the cylinder. 2d. Recall that dilution water is distilled water plus the calcium, magnesium, ferric, and buffer solutions.	
	3. Thoroughly mix the contents of the cylinder.	3a. Use a plunger type mixer. 3b. Cause no splashing of the liquid.	
	4. Repeat steps 1., 2., and 3., except use 150 ml of seed and a second cylinder.	4a. Use a 100 ml and a 50 ml volumetric pipet.	
	5. Repeat steps 1., 2., and 3., except use 200 ml of seed and a third cylinder.	5a. Use a 100 ml volumetric pipet.	
	6. Calculate the % of seed in each of the 3 graduated cylinders.	6a. $\frac{100}{1000} \times 100 = 10$ % of seed in the first cylinder. 6b. $\frac{150}{1000} \times 100 = 15$% of seed in the second cylinder. 6c. $\frac{200}{1000} \times 100 = 20$% of seed in the third cylinder.	

EFFLUENT MONITORING PROCEDURE: Dechlorination of Samples for Biochemical Oxygen Demand and Seeding of the Dilution Water

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Calculation of Oxygen Depletion in the Sample, Due to Seed (continued)			
	7. Fill 2 BOD bottles from the first (10%) cylinder by siphoning.	7a. Hold the end of the siphon near the bottom of the BOD bottle so as to prevent splashing. 7b. Open the siphon slowly. 7c. Fill the bottles until the liquid just begins to overflow.	
	8. Stopper the 2 BOD bottles tightly and label them.	8a. Do not cause formation of an air bubble by inserting the stopper too vigorously.	
	9. Repeat steps 7 and 8 using the second (15%) cylinder and 2 more BOD bottles.		
	10. Repeat steps 7 and 8 using the third (20%) cylinder and 2 more BOD bottles.		
	11. Fill the flared top of 3 of the stoppered bottles with water.	11a. One of the 10%, one of the 15%, and one of the 20% bottles.	
	12. Store them in the incubator for 5 days.	12a. At $20^{o}C \pm 1^{o}C$. 12b. In the dark. 12c. Check the flared tops at least twice daily and refill with water as needed.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Calculation of Oxygen Depletion in the Sample, Due to Seed (continued)			
2. Determination of Oxygen Depletion in the Seed Controls	1. Determine and record the initial DO of the other 3 bottles.	1a. The other 10%, 15%, and 20% bottles. 1b. Use the Winkler Method - azide modification, or a DO meter. See the appropriate effluent monitoring procedure. 1c. The DO determination should be made within 15 minutes after mixing the contents of the liter graduated cylinder.	
	2. After five days, determine and record the final DO of of the 3 incubated bottles.	2a. Use the same method as for the initial DO determinations. 2b. Example calculation: 7.0 mg/l = initial DO of the 20% seed control 2.8 mg/l = final DO of the 20% seed control 4.2 mg/l = 5 day DO depletion of the 20% seed control $\frac{4.2}{7.0}$ X 100 = 60% DO depletion in the 20% seed control. (This is within the 40-70% range.) By similar calculation, if the 15% seed control gives a 40% depletion, use the 20% seed control for calculating the final BOD$_5$ value, as explained before. 2c. The 20% seed control will be used for the rest of the example calculations.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Calculation of Oxygen Depletion in the Sample, due to Seed (continued)			
3. Seeding of the Dilution Water	1. Pipet the calculated amount of seed (supernatant liquid) from the 2 liter flask (B.1.2.). This is the same seed used to prepare the seed controls in F.1.	1a. For example, assume 3 liters of dilution water are left after preparation of the seed controls (F.1.). 1b. Two ml of the supernatant liquid (seed) are used for each 1 liter of dilution water - seed mixture. 1c. Continuing the example, 6 ml of seed would be pipetted (3 x 2). 1d. As mentioned in the effluent monitoring procedure on the Determination of Five-Day Biochemical Oxygen Demand, during the incubation period, at least 2 mg of dissolved oxygen/l must be used by the sample, and at least 1 mg/l must remain at the end of the incubation period. If less than the 2 mg/l is used, increase the amount of seed; e.g., maybe 4 or 5 ml of seed will be needed for each liter of the dilution water-seed mixture. Only experience can determine the proper amount of seed to use. 1e. Use a graduated pipet to measure the seed. Ten ml size in the example. If solids clog the tip of the pipet, use a graduated cylinder. Ten ml size in the example.	
	2. Drain the seed from the pipet into the dilution water container.	2a. Continuing the example, there are now 3006 ml of liquid in the container.	
	3. Mix the dilution water and seed by swirling the container.	3a. After mixing, the dilution water is said to be seeded.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Calculation of Oxygen Depletion in the Sample, due to Seed (continued)	4. Place the container of seeded dilution water in the incubator.	4a. 20°C ± 1°C. 4b. Until it is needed.	
	5. The 2 liter flask may now be emptied.		
4. Determination of Seed Control Correction	1. Determine what dilutions will be made on the sample.	1a. See the effluent monitoring procedure on the Determination of Five-Day Biochemical Oxygen Demand, sections C.1.1e. and C.1.1f. 1b. For the purpose of example, assume you have gained experience about the proper sample volume to use, and you are going to set up a 40% dilution on the effluent of a treatment plant treating domestic wastewater.	
	2. Calculate the amount of seeded dilution water needed.	2a. Continuing the example: the dilution is being done in a 1 liter graduated cylinder. 2b. 1000 x 0.40 (40% as a decimal) = 400 ml sample. 2c. 1000 - 400 = 600 ml dilution water.	
	3. Calculate the amount of seed present in the dilution water used to dilute the sample.	3a. Continuing the example: 600 ml of dilution water are being used. 3b. 6 ml of seed were added to 3 liters of dilution water; i.e., 6 ml seed, and 3000 ml dilution water. 3c. $\frac{6}{6 + 3000} \times 100 =$ 0.2% seed in the seeded dilution water	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Calculations of Oxygen Depletion in the Sample, Due to Seed (continued)	4. Calculate the % of seed material in the 300 ml BOD bottles containing the sample.	4a. Continuing the example: A 40% dilution of the sample is being set up (as was mentioned in F.4.1.1b.). 4b. In the 300 ml BOD bottle: 40% = sample 60% = seeded dilution water 4c. 300 X 0.4 (40% as a decimal) = 120 ml sample 300 X 0.6 (60% as a decimal) = 180 ml seeded dilution water. The seeded dilution water contains 0.2% seed (F.4.3.3c. above). 180 X 0.002 (0.2% as a decimal) = 0.36 ml seed material in the 300 ml BOD bottles. 4d. $\frac{0.36}{300} \times 100 = 0.12\%$ seed in the BOD bottle	
	5. Calculate the % of seed in the seed control.	5a. Continuing the example: The 20% seed control gave a 60% oxygen depletion and will be used for the example calculation as mentioned in F.2.2.2b. and F.2.2.2c. 5b. Therefore, there is 20% seed in the seed control.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Calculations of Oxygen Depletion in the Sample, Due to Seed (continued)	6. Calculate the seed control correction factor.	6a. Continuing the example: $\frac{\text{\% seed in BOD bottle}}{\text{\% seed in the seed control}}$ $= \frac{0.12}{20}$ $= 0.006$	
	7. Calculate the seed correction.	7a. Continuing the example: seed correction = five day oxygen depletion in seed control X factor. 7b. Oxygen depletion = 4.2 mg/l (from F.2.2.2b.) X 0.006 = 0.025 mg/l	
G. Calculation of BOD_5, Corrected for Use of Seeded Dilution Water	1. Calculate mg BOD_5/l for the sample.	1a. See the effluent monitoring procedure on the Determination of Five-Day Biochemical Oxygen Demand, section D.1. 1b. Continuing the example: Assume the BOD_5 to be 30 mg/l.	
	2. Subtract the seed correction.	2a. Continuing the example: mg BOD_5/l = 30 mg/l - 0.025 mg/l = 29.98 mg/l = 30 mg/l (rounded off)	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
G. Calculation of BOD_5, Corrected for Use of Seeded Dilution Water (continued)		2b. In this example, the seed correction was small enough to be ignored. However, it will not necessarily always be small enough to be ignored.	

TRAINING GUIDE

SECTION	TOPIC
I	Introduction
II	Educational Concepts - Mathematics
III	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field and Laboratory Equipment
VI	Field and Laboratory Reagents
VII	Field and Laboratory Analysis
VIII	Safety
IX	Records and Reports

*Training guide materials are presented here under the headings marked *.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.1.1	If the glassware is especially dirty and cannot be cleaned with ordinary detergents, chromic acid cleaning may be required. 1. Pour 35 ml of distilled water in a 250 ml beaker. 2. Add about 1/8 teaspoon (simply estimate this quantity) of sodium dichromate, $Na_2Cr_2O_7$, to the water. 3. Swirl the beaker until the sodium dichromate has dissolved. 4. Keep repeating steps 2 and 3 until no more sodium dichromate will dissolve. 5. Pour the solution into a 2 liter beaker. 6. Slowly pour 1 liter of concentrated sulfuric acid, H_2SO_4, into the 2 liter beaker. CAUTION: Use eyeglasses and protective clothing. 7. Stir the mixture thoroughly. 8. Store it in a glass stoppered bottle. 9. The cleaning solution should be at a temperature of about 50°C when it is used. 10. It may therefore be necessary to warm the cleaning solution. 11. When using the warm cleaning solution, fill the piece of glassware with the solution. 12. Allow it to soak for 2-3 minutes (or longer). 13. Pour the cleaning solution back into the storage bottle. 14. Rinse the piece of glassware ten times with tap water. 15. The cleaning solution may be reused until it turns green. 16. It should then be discarded.	

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

DETERMINATION OF DISSOLVED OXYGEN USING A DISSOLVED OXYGEN METER

as applied in

WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

Developed by the

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

100

EFFLUENT MONITORING PROCEDURE: Determination of Dissolved Oxygen Using A Dissolved Oxygen Meter

This process was developed by:

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EDUCATION AND TECHNICAL BACKGROUND

B.S. - Chemistry

M.S. - Chemistry

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4 years college Chemistry Instructor

1-1/2 years DHEW - Air Pollution Program, Chemist

10 years DI - EPA, Chemist-Instructor

EFFLUENT MONITORING PROCEDURE: Determination of Dissolved Oxygen Using A Dissolved Oxygen Meter

1. Analysis Objectives:

The learner will use the attached EMP to place the Weston and Stack Model 300 Dissolved Oxygen Meter into operation, including electrode cleaning, membrane installation, calibration, and use of the meter to make a dissolved oxygen measurement.

2. Brief Description of Analysis:

The Winkler determination of dissolved oxygen-azide modification is subject to many interferences. In the case of a BOD_5 determination, the problem is minimized to some extent because of sample dilution. If it is felt, however, that appreciable amounts of interfering materials are present, a dissolved oxygen meter should be used.

3. Applicability of this Procedure:

At concentrations normally found in wastewater effluents, chlorine does not affect the dissolved oxygen probe. Prolonged exposure to higher concentrations of chlorine, and hydrogen sulfide, will necessitate cleaning of the lead anode. Oil and grease will coat the membrane causing a decrease in sensitivity; the membrane should be replaced in this case.

This procedure was excerpted from the instruction book supplied with the meter by the manufacturer.

Mention of a particular brand name does not constitute endorsement by the U.S. Environmental Protection Agency

EFFLUENT MONITORING PROCEDURE: Determination of Dissolved Oxygen Using a Dissolved Oxygen Meter

General Description of Equipment Used in a Process

A. Capital

1. Weston and Stack Model 300 Dissolved Oxygen (DO) Meter with Model A-30 Probe, accessory kit and manufacturers instruction book
2. Still, or other source of distilled water
3. Trip balance, 100 g. capacity

B. Reusable

1. One 100 ml graduated cylinder
2. One 250 ml Erlenmeyer Flask
3. One 100 ml glass stoppered bottle
4. One 200 ml plastic bottle
5. One teaspoon
6. Small blade screwdriver
7. One 250 ml beaker
8. Five ml syringe or eyedropper with tapered end
9. Small pocket knife
10. Four 300 ml BOD bottles
11. Equipment for performing a Winkler DO determination-azide modification, see the effluent monitoring procedure Winkler Determination of Dissolved Oxygen-Azide Modification.

C. Consumable

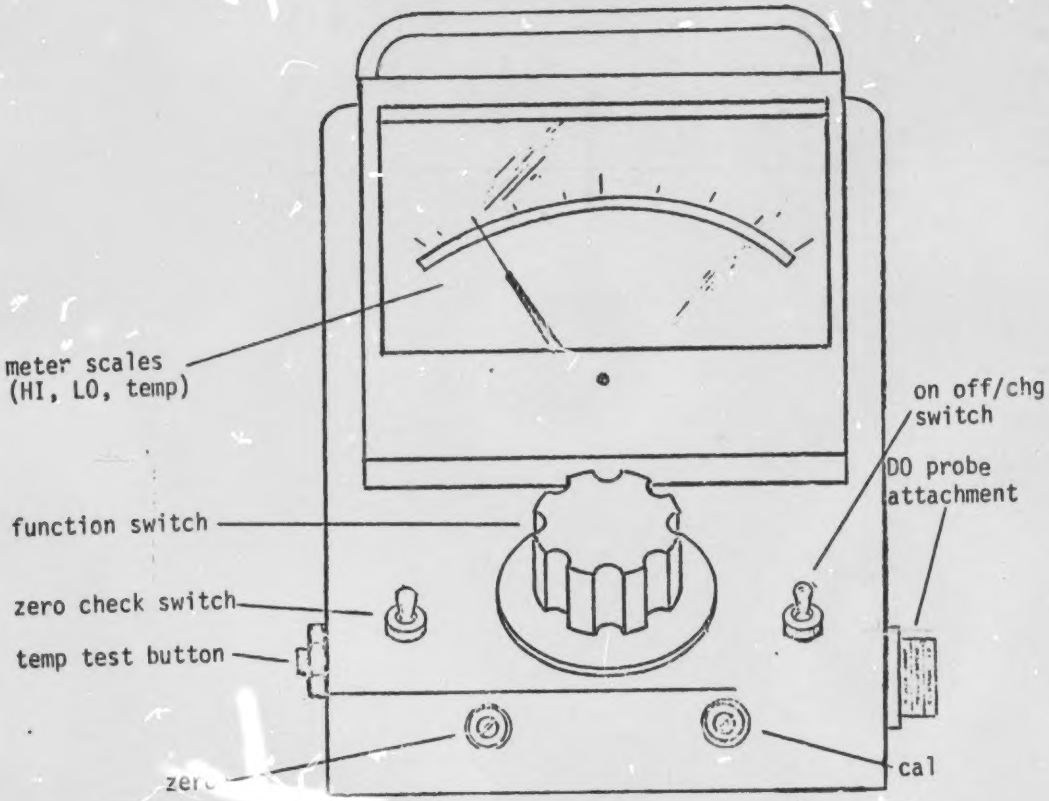
1. Potassium iodide, KI, 50 g.
2. Sodium sulfite, Na_2SO_3, 25 g.
3. Sodium hydroxide, NaOH, 10 g.
4. One rubber band
5. Paper towels
6. Silicone lubricant
7. Source of distilled water
8. One 1 inch long piece of scotch tape
9. Reagents for performing a Winkler DO determination-azide modification, see the effluent monitoring procedure Winkler Determination of Dissolved Oxygen-Azide Modification.
10. Sodium dichromate, $Na_2Cr_2O_7$
11. Concentrated sulfuric acid, H_2SO_4
12. Soap
    (Items 10, 11, and 12 are for cleaning glassware. The quantities will therefore vary.)
13. Brushes (for cleaning glassware)
14. Brush (for cleaning balance)
15. Sponges (for cleaning of laboratory table tops)

C. Continued

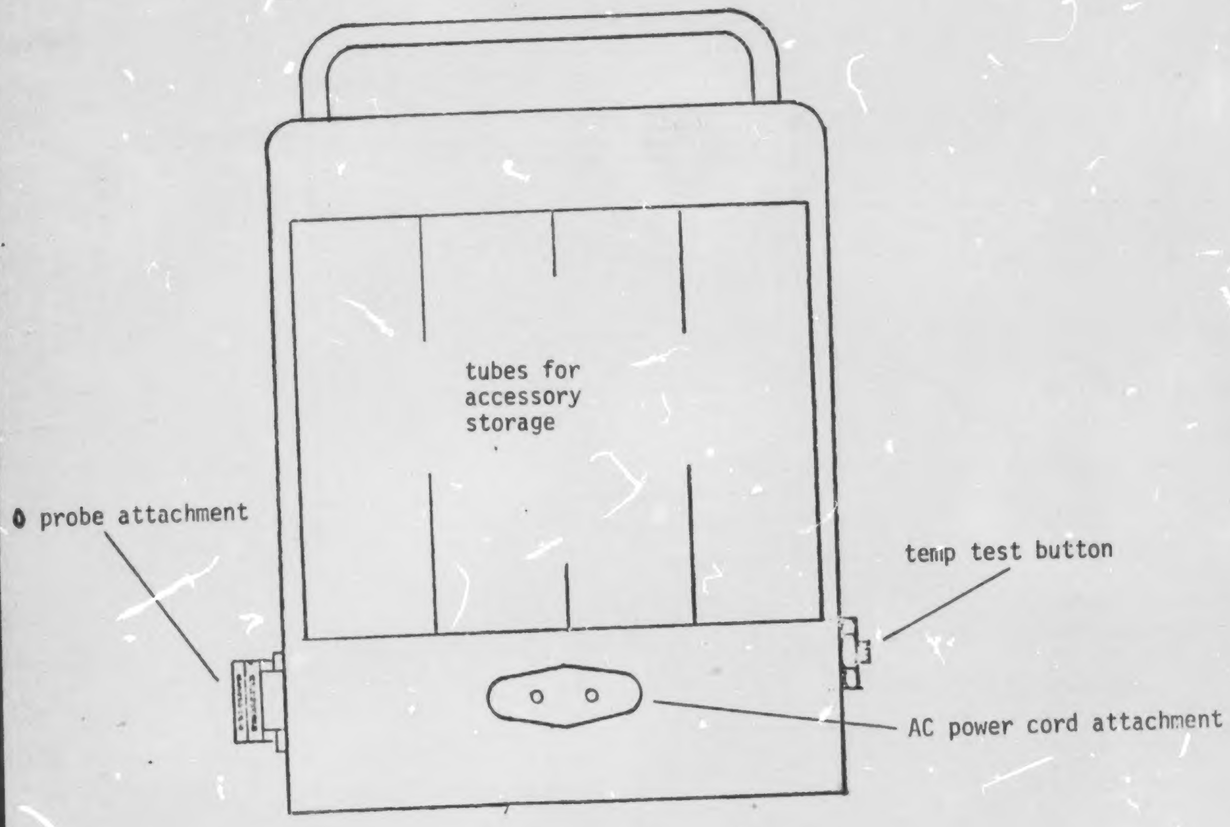
All reagents should be of high quality. Different chemical manufacturers may have different ways of indicating a high quality reagent. While no endorsement of one chemical manufacturer over another is intended, the following are some designations used in four chemical catalogs to indicate high quality reagents.

Catalog	Designations
Thomas	Reagent, ACS, Chemically Pure (CP)
Matheson, Coleman & Bell	Reagent, ACS
Curtin Matheson Scientific, Inc.	Primary Standard, ACS, AR
Fisher	Certified, ACS

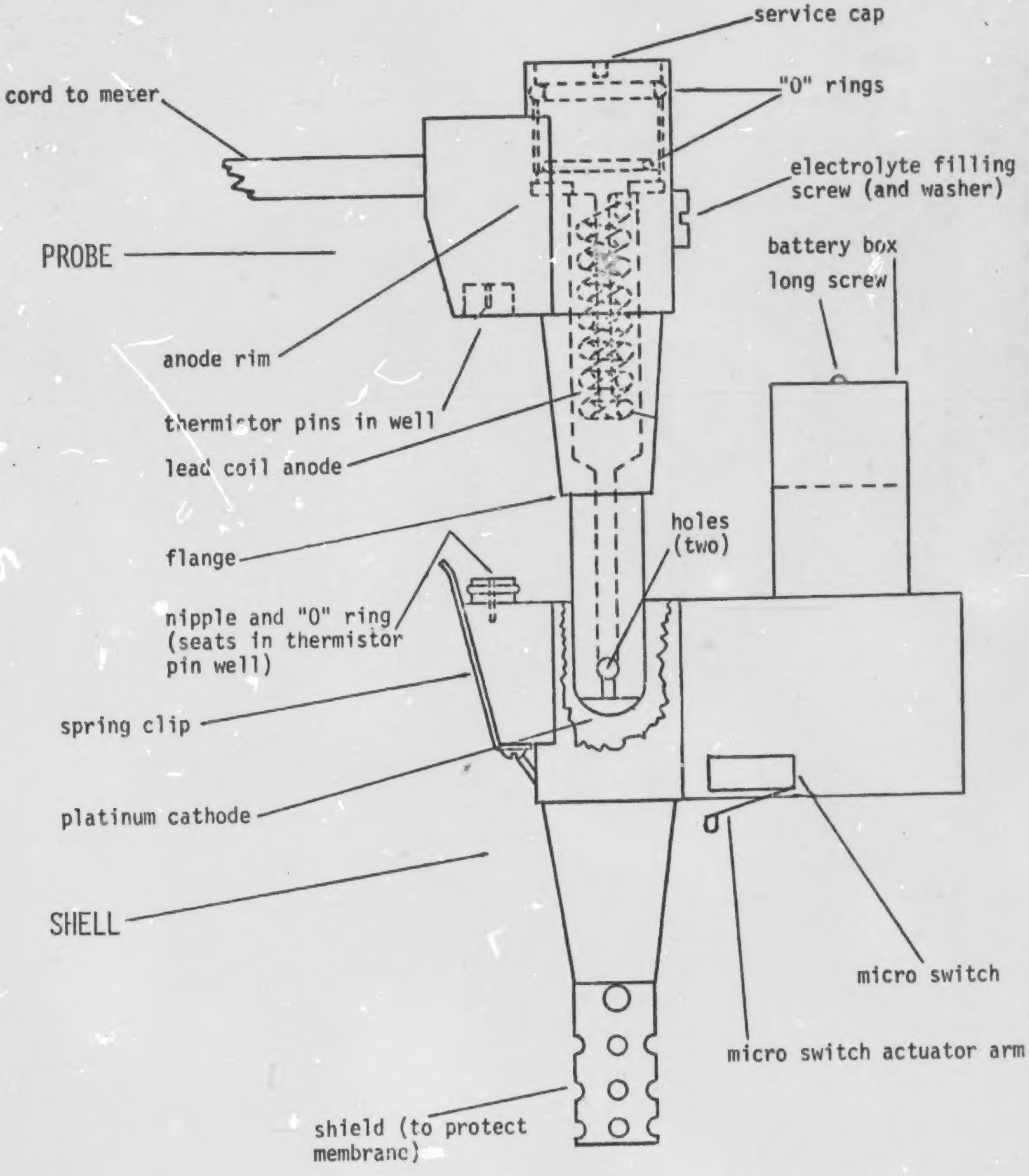
FRONT VIEW OF METER


meter scales
(HI, LO, temp)
function switch
zero check switch
temp test button
zero
on off/chg
switch
DO probe
attachment
cal

REAR VIEW OF METER


tubes for
accessory
storage
0 probe attachment
temp test button
AC power cord attachment

CUT-AWAY VIEW OF PROBE, SHELL, AND STIRRING MECHANISM


service cap
cord to meter
"O" rings
electrolyte filling screw (and washer)
PROBE
battery box
long screw
anode rim
thermistor pins in well
lead coil anode
flange
holes (two)
nipple and "O" ring (seats in thermistor pin well)
spring clip
platinum cathode
SHELL
micro switch
micro switch actuator arm
shield (to protect membrane)

107

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Equipment Preparation 1. Glassware	1. Clean all glassware and rinse with distilled water.		V.A.1.1 (p. 22)
2. Balance inspection	1. Check all balances for cleanliness and proper operation.		
B. Reagent Preparation 1. Electrolyte solution	1. Weigh 50 g. of potassium iodide, KI.		
	2. Weigh 0.1 g. of sodium sulfite.		
	3. Dissolve the two solids in 100 ml of water.	3a. Unless otherwise specified, the term water means distilled water. 3b. Unless otherwise specified, solutions should be stored in glass stoppered bottles.	
	4. Store the electrolyte in a small bottle.		
2. Sodium hydroxide solution	1. Weigh 10 g. of sodium hydroxide, NaOH.		
	2. Dissolve it in 90 ml of water.		
	3. Store the solution in a small plastic bottle.		
3. Sodium sulfite solution	1. Measure 1 teaspoon of sodium sulfite, Na_2SO_3.		
	2. Dissolve it in 500 ml of tap water.	2a. Prepare this solution just prior to use.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Equipment Preparation 1. Battery check - Weston and Stack Model 300 Dissolved Oxygen (DO) Meter	1. Check the power cord attached to the rear of the meter.	1a. The meter is portable and the cord should be plugged in to recharge the nickel cadmium battery during storage periods.	
	2. Turn the function switch to the temperature position.		
	3. While pressing the temp test button (left side of meter), adjust the temp adj screw (right side of meter) to read 50°C (bottom scale on front of meter).		
	4. Turn the function switch to the transit position.		
2. Battery check-DO probe stirring mechanism	1. Remove the long screw from the top of the battery box.		
	2. Remove the top half of the battery box.		
	3. Insert two size AA 1-1/2 volt batteries (provided with the instrument) into the battery box.	3a. One should be upright, the other upside down.	
	4. Place the top on the battery box.	4a. Note that there is a tip and a hole on the bottom of the top half of the battery box. These fit into a hole and a tip on the top of the bottom half of the battery box.	
	5. Insert the long screw into the top of the battery box.		
	6. Screw it down.		
	7. Using your finger, close the micro switch actuator arm on the side of the probe.	7a. The probe stirring mechanism will start.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
2. Continued	8. Loosen the long screw.		
	9. Raise the top half of the battery box about half inch.		
	10. Wrap a rubber band around the battery box.	10a. The rubber band should be placed in such a way that the two halves of the battery box are kept apart. 10b. This will keep the stirring mechanism from operating when the probe is not in use. 10c. Do not remove the rubber band and tighten the long screw until the meter is to be calibrated or measurements are to be made.	
3. Cathode check-DO probe	1. Unfasten the spring clip and remove the probe from the shell.		
	2. Examine the platinum cathode at the end of the probe.	2a. It should be free of dirt. 2b. If it is not, wipe it briskly with a paper towel, or coarse piece of cloth.	
4. Anode check-DO probe	1. Remove the service cap on top of the probe.		
	2. Examine the two black "O" rings.	2a. They should be free of dirt.	
	3. Coat the two "O" rings with a very thin layer of silicone lubricant.		
	4. Invert the probe over a table top.	4a. The lead coil anode should drop out. 4b. If it does not, tap the probe lightly on the table top.	

EFFLUENT MONITORING PROCEDURE: Determination of Dissolved Oxygen Using a Dissolved Oxygen Meter

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
4. Continued	5. Examine the anode.	5a. It should be free of dirt and corrosion. Yellow colored corrosion is common.	
	6. If corrosion is present, soak the anode in the sodium hydroxide solution.	6a. A few minutes soaking should suffice. 6b. Very minute amounts of corrosion will not cause problems.	
	7. Rinse the anode thoroughly with tap water.		
	8. Rinse the anode thoroughly with distilled water.		
	9. Examine the rim, inside of the probe, on which the anode sits.	9a. It should be free of dirt and corrosion. Yellow colored corrosion is common.	
	10. If corrosion is present, scrape it away using the blade of a small screwdriver.	10a. A swab dipped in the sodium hydroxide solution will also remove the corrosion.	
	11. Rinse the rim and interior of the probe thoroughly with tap water and then with distilled water.		
5. Thermistor contact check	1. Invert the probe.		
	2. Examine the two thermistor pins in the small well.	2a. They should be free of dirt and corrosion. 2b. If they are corroded, gently scrape them, using the blade of a small screwdriver.	
	3. Examine the "O" ring around the nipple which seats in the thermistor pin well.	3a. It should be free of dirt.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
5. Continued	4. Coat it with a very thin layer of silicone lubricant.		
6. Membrane installation	1. Select a 1 mil membrane (furnished with the instrument).	1a. A mil is 0.001 inch. 1b. One-half mil membranes are sometimes used. They respond faster, but are more fragile.	
	2. Examine it in bright light for holes.	2a. If any are seen, discard the membrane.	
	3. Hold the probe upside down.		
	4. Lay the square membrane over the platinum cathode.	4a. The cathode should be in the center of the square.	
	5. Fold the membrane back over the probe.		
	6. Loop a small rubber band (furnished with the instrument) around the probe three times just above the flange.	6a. The rubber band should hold the membrane snugly, but not too tightly. 6b. Two turns of the rubber band may suffice in some cases.	
	7. Gently pull on the loose edges of the membrane.	7a. There should be no folds in the membrane over the platinum cathode. 7b. The pulling should not, however, cause tearing of the membrane.	
	8. Place a short piece of scotch tape over the well containing the two thermistor pins.	8a. This will prevent moisture from getting into the thermistor pin well while the probe is being filled with electrolyte.	
	9. Remove the electrolyte filling screw and washer.		
	10. Hold the probe in a vertical position, with a finger tip held over the electrolyte filling well.	10a. The cathode should point down.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
6. Continued	11. Pour electrolyte solution into the service cap opening.	11a. Fill the probe interior almost to the top.	
	12. With a twisting motion, slide the rubber band and membrane down about 1/2 inch.	12a. The pocket formed by the membrane will fill with electrolyte.	
	13. With a similar motion, slide the rubber band and membrane back up to its original position.	13a. There should be no wrinkles in the part of the membrane lying across the platinum cathode.	
	14. Loop a second rubber band around the membrane about half-way between the first rubber band and the two holes near the platinum cathode.	14a. Wrap the rubber band as tightly as possible, one turn next to another. 14b. There should still be no wrinkles in the part of the membrane lying across the platinum cathode. 14c. It may be awkward to keep a finger tip over the electrolyte filling well. 14d. No problem is created if some electrolyte is lost, it will be replaced later.	
	15. Carefully drop the lead anode into place.		
	16. Screw in the service cap about half-way.		
	17. Hold the probe in a horizontal direction, electrolyte filling hole up.		
	18. Remove the finger tip from the electrolyte filling well.	18a. Some electrolyte will probably run out.	
	19. Using a syringe or eyedropper with a tapered end, add additional electrolyte through the filling hole.	19a. Gently rock the probe back and forth after each addition so as to dislodge air bubbles. 19b. Tap the sides of the probe with a pen or pencil to ensure bubble escape.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
6. Continued	20. Tighten the service cap.		
	21. Check for completeness of electrolyte filling.		
	22. Replace the electrolyte filling screw and washer.		
	23. Ensure that no air bubbles have formed under the membrane covering the platinum cathode.	23a. If there are any, the membrane should be removed and reinstalled.	
	24. Cut the membrane around the probe between the two rubber bands.		
	25. Cut away the first rubber band.		
	26. Remove the excess membrane.		
	27. Carefully rinse the outside of the probe and membrane with water.		
	28. Gently shake off the water.		
	29. Remove the piece of tape covering the thermistor pin well.		
	30. Carefully place the probe back in the shell.	30a. Be cautious not to tear the membrane. 30b. Make sure the spring clip is properly closed.	
	31. Place the probe in a 300 ml BOD bottle full of water.		

EFFLUENT MONITORING PROCEDURE: Determination of Dissolved Oxygen Using a Dissolved Oxygen Meter

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
6. Continued	32. With your eyes at the same level as the end of the probe, look carefully at the end of the probe for about one minute.	32a. If there are any holes in the membrane the leaking electrolyte will be seen in the water, and the membrane must be replaced.	
7. DO meter zeroing	1. Attach the DO probe to the DO meter.	1a. A pliers may be used to assure a snug fit, but be careful not to damage the knurls on the locking collar.	
	2. Rinse the outside of the shell with water.	2a. Do not get water on the micro switch of the stirring mechanism.	
	3. Place the probe in a 300 ml BOD bottle filled with sulfite solution.		
	4. Allow it to stand for 10 minutes.		
	5. Remove the rubber band from the stirring mechanism battery box.		
	6. Screw down the long screw in the top of the battery box.	6a. The stirring mechanism should start since the flared top of the BOD bottle closes the micro switch actuator arm.	
	7. Wait two minutes.		
	8. Turn the on off/chg toggle switch to the on position.		
	9. Turn the function switch to the HI mg/liter position.	9a. The needle on the meter face will move to the right and then slowly drift to the left.	
	10. When the needle reads 1.5 on the top scale, turn the function switch to the LO mg/liter position.	10a. The needle should continue to drift to the left.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
7. Continued	11. Wait one additional minute.		
	12. Depress the zero check toggle switch and turn the zero screw (bottom front of the instrument) until the needle reads 0 on the middle scale of the meter.	12a. After releasing the toggle switch the needle may slowly drift toward a "true" zero.	
	13. Turn the on off/chg toggle switch to the off/chg position.	13a. In the off/chg position, the nickle cadmium battery is charging when the power cord is attached.	
	14. Turn the function switch to the transit position.		
	15. Loosen the long screw and raise the top half of the battery box.	14a. For the remainder of this EMP, this procedure will be referred to as turning the stirring mechanism off. Lowering the top half of the battery box and tightening the long screw will be referred to as turning the stirring mechanism on.	
	16. Replace the rubber band which separates the two halves of the battery box.		
	17. Remove the probe from the BOD bottle.		
	18. Rinse off the bottom of the probe thoroughly.	17a. Be cautious not to get the micro switch wet. 17b. All traces of the sulfite solution must be removed.	
	19. Place the probe in a BOD bottle filled with water.	18a. To prevent the membrane from drying out, always keep the probe in water when not in use.	
8. DO meter calibration	1. Fill two 300 ml BOD bottles to overflowing with distilled water.	1a. It is essential that the two samples be identical in oxygen content.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
8. Continued	2. Determine the DO of one of the bottles.	2a. Use the Winkler Method-Azide Modification.	
	3. Remove the probe from the BOD bottle containing the water.		
	4. Rinse the end of the probe with distilled water.		
	5. Place the probe in the second BOD bottle filled with distilled water.		
	6. Turn the on off/chg toggle switch to the on position.		
	7. Turn the stirring mechanism on.		
	8. Turn the function switch to the HI mg/liter position.		
	9. Wait about two minutes.	9a. Because the slower responding 1 mil membrane is being used, about two minutes should be required for the needle reading to stabilize. 9b. With some meters, experience may indicate that a shorter waiting period will suffice.	
	10. Turn the cal screw (bottom front of the meter) until the needle reads (on the upper scale) the same DO value as was obtained from the Winkler titration.		
	11. Turn the stirring mechanism off.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
8. Continued	12. Turn the on off/chg toggle switch to the off/chg position.		
	13. Turn the function switch to the transit position.	13a. There is no firm rule about how often to zero and calibrate the Weston and Stack Model 300 DO meter. 13b. A conservative estimate would be to do the calibration daily, and the zeroing every other day in times of frequent use. 13c. Both steps should be performed if the meter has not been used for several days. 13d. After installation of a new membrane, the calibration changes markedly during the first 24 hours, and frequent calibrations are needed during this period.	
	14. Place the probe back in the BOD bottle of distilled water. The membrane should always be kept wet when not in use.		

TRAINING GUIDE

SECTION	TOPIC
I	Introduction
II	Educational Concepts - Mathematics
III	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field & Laboratory Equipment
VI	Field & Laboratory Reagents
VII	Field & Laboratory Analysis
VIII	Safety
IX	Records & Reports

*Training guide materials are presented here under the headings marked *.

TRAINING GUIDE NOTE

REFERENCES/RESOURCES

A.1.1

If the glassware is especially dirty and cannot be cleaned with ordinary detergents, chromic acid cleaning may be required.

1. Pour 35 ml of distilled water in a 250 ml beaker.
2. Add about 1/8 teaspoon (simply estimate this quantity) of sodium dicrhomate, $Na_2Cr_2O_7$ to the water.
3. Swirl the beaker until the sodium dichromate has dissolved.
4. Keep repeating steps 2 and 3 until no more sodium dichromate will dissolve.
5. Pour the solution into a 2 liter beaker.
6. Slowly pour 1 liter of concentrated sulfuric acid, H_2SO_4, into the 2 liter beaker.

   Caution: Use eyeglasses and protective clothing.
7. Stir the mixture throughly.
8. Store it in a glass stoppered bottle.
9. The cleaning solution should be at a temperature of about 50°C when it is used.
10. It may therefore be necessary to warm the cleaning solution.
11. When using the warm cleaning solution, fill the piece of glassware with the solution.
12. Allow it to soak for 2-3 minutes (or longer).
13. Pour the cleaning solution back into the storage bottle.
14. Rinse the piece of glassware ten times with tap water.
15. The cleaning solution may be reused until it turns green.
16. It should then be discarded.

13th Standard Methods, p. 135, section 2.c.2

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

DETERMINATION OF DISSOLVED OXYGEN IN WASTEWATER: POLAROGRAPHIC PROBE METHOD

as applied in

WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

Developed by the

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

EFFLUENT MONITORING PROCEDURE: Determination of Dissolved Oxygen: Polarographic Probe Method (YSI Model 54 Oxygen Meter)

This instructional sequence was prepared by:

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EDUCATION & TECHNICAL BACKGROUND

BS - Chemistry

14 years Industrial Chemist

16 years HEW-FWPCA-EPA-Chemist

EFFLUENT MONITORING PROCEDURE: Determination of Dissolved Oxygen: Polarographic Probe Method (YSI Model 54 Oxygen Meter)

1. Analysis Objectives:

The operator will be able to set up, calibrate and use a YSI oxygen meter for the determination of dissolved oxygen in a sample of wastewater treatment plant effluent.

2. Brief Description of Analysis*:

The meter is set up and calibrated and the polarographic probe is inserted into the appropriate sample. A reading is obtained from the meter which correlates the dissolved oxygen concentration in the sample.

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*Standard Methods for the Examination of Water and Wastewater, 14th Ed., 1975. APHA, Washington, DC, p. 450

EFFLUENT MONITORING PROCEDURE: Determination of Dissolved Oxygen: Polarographic Probe Method (YSI Model 54 Oxygen Meter)

General Description of Equipment Used in the Process

A. Capital Equipment

1. Dissolved oxygen meter and polarographic probe assembly - Yellow Springs Instrument Company

B. Reusable

1. B.O.D. bottle (300 ml)
2. One plastic squeeze bottle
3. Eyedropper bottle
4. Scissors
5. Small screwdriver

C. Consumable

1. Standard membranes (0.001" - YSI #5352)
2. Probe Service Kit (YSI #5034)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Probe Preparation	1. Add distilled water to the KCl crystals and dissolve completely.	1a. KCl crystals included in YSI Probe Service Kit (Part no. 5034) a saturated KCl solution diluted 1:1 with distilled water should be used.	
	2. Transfer a part of the KCl solution to the eyedropper bottle.		
	3. Remove the protective membrane and "O" ring.		
	4. Select a membrane from the membrane packet.	4a. Lay on a clean sheet of paper. Handle only by the ends. 4b. Use only YSI recommended membranes and filling solution. Distilled water must be used in making the KCl solution. Tap water contains iron and other salts that result in poor electrode performance and will contaminate the electrodes and result in short life.	
	5. Support the probe in a vertical position.		
	6. With one thumb secure one end of the membrane to the side of the probe.		
	7. With the eyedropper, fill the central hole avoiding air bubbles.		
	8. Wet the gold electrode and the lucite around it.	8a. The surface tension of the KCl will cause a large drop or meniscus to form above the electrode. This will ensure complete contact between the membrane and the KCl.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Continued	9. Stretch the membrane over the top of the electrode.		
	10. Stretch an "O" ring into place--inspect for wrinkle-free membrane.	10a. A taut smooth membrane surface is required. A lax membrane will result in erratic performance, slow speed of response and poor shock performance.	
	11. Remove the excess membrane about 1/8" beyond the "O" ring with scissors.		
	12. A small air bubble may appear under the membrane.	12a. This is normal, however, strive for a bubble-free probe. New probes, or probes that have been allowed to dry out, will continue to develop bubbles until the porous anode is completely filled.	
	13. After the air has been driven from the anode, remove the membrane, refill with KCl, and install another membrane.		
	14. Rinse probe with distilled water.		
	15. The probe is ready for operation.		
B. Calibration	1. Connect the two probe plugs to the jacks on the side of the instrument.		
	2. With the instrument turned off check the mechanical zero of the meter--pointer should indicate zero.	2a. Adjust with the screw on the meter case. Recheck when the position of instrument is changed.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Continued	3. Switch to the RED LINE position and adjust the meter to red line with the front panel control.		
	4. Place the probe in the BOD bottle containing a water sample of known dissolved oxygen content.	4a. Samples of known oxygen concentration can be obtained by analyzing a duplicate sample by the Winkler Titration Method.	
	5. Turn the stirring mechanism switch on.		
	6. Switch to the TEMP position and read the temperature when the meter is steady.		
	7. Switch to the ZERO position and adjust the meter to zero with the ZERO control.		
	8. Switch to the 0-10 ppm position and calibrate the instrument with the CAL control.	8a. Calibration and measurement should be carried out on the same range to avoid compounding meter tolerance error.	
	9. Turn off the stirring mechanism and the instrument.		
C. Dissolved Oxygen Measurement	1. Place the probe in the BOD bottle containing the unknown sample.		
	2. Turn on the stirring mechanism and the instrument.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Continued	3. Switch to the 0-10 ppm position and read the dissolved oxygen concentration obtained. 4. Turn off the stirring mechanism and the instrument.		

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

pH DETERMINATION OF WASTEWATER AND WASTEWATER TREATMENT PLANT EFFLUENTS

as applied in

WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

Developed by the

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

EFFLUENT MONITORING PROCEDURE: Measurement of pH

This instructional sequence was developed by:

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EDUCATION AND TECHNICAL BACKGROUND

B.S. Chemistry

14 years Industrial Chemist

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EFFLUENT MONITORING PROCEDURE: Measurement of pH

1. Analysis Objectives:

   WWTP operator will set up, calibrate and operate portable type pH meter for the pH measurement of wastewater and WWTP effluent.

2. Brief Description of Analysis*

   A portable type, battery operated pH meter, equipped with a glass electrode system is used to measure the pH of wastewater treatment plant samples.

3. Applicability of this Procedure:

   a. Range of concentrations:

      pH scale 0-14

   b. Pretreatment of Sample:

      None

   c. Treatment of interferences in samples:

      None

---

*Standard Methods for the Examination of Water and Wastewater, 14th Ed., 1975, APHA, Washington, D.C., p. 460

EFFLUENT MONITORING PROCEDURE: Measurement of pH

General Description of Equipment Used in the Process

A. Capital Equipment
  1. pH Meter IL Model 175 PORTO-matic*
     The IL Model 175 PORTO-matic pH meter is a small, solid state, battery operated, portable instrument for the measurement of the pH of aqueous solutions. Manufacturer's Specifications are as follows:

     pH range: 0-14

     pH Scale: 7.2", 1/2% zero centered

     Readability: 0.01 pH

     Electrical Accuracy: better than 0.035 pH

     Drift per Day: less than 0.01 pH

     Battery Life: 2000 hours

B. Reusable
  1. Wash bottle, plastic
  2. Beakers, 250 ml, 150 ml, 25 ml

C. Consumable
  1. Buffer Solution pH 4
  2. Buffer Solution pH 9
  3. Buffer Solution pH 6.9
  4. Buffer Solution pH 7.4
  5. Saturated KCl Solution

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*Mention of a specific brand name does not constitute endorsement by the U.S. Environmental Protection Agency

EQUIPMENT - PORTO-MATIC pH METER

OPERATING CONTROLS FRONT PANEL, 175 PORTO-MATIC pH METER

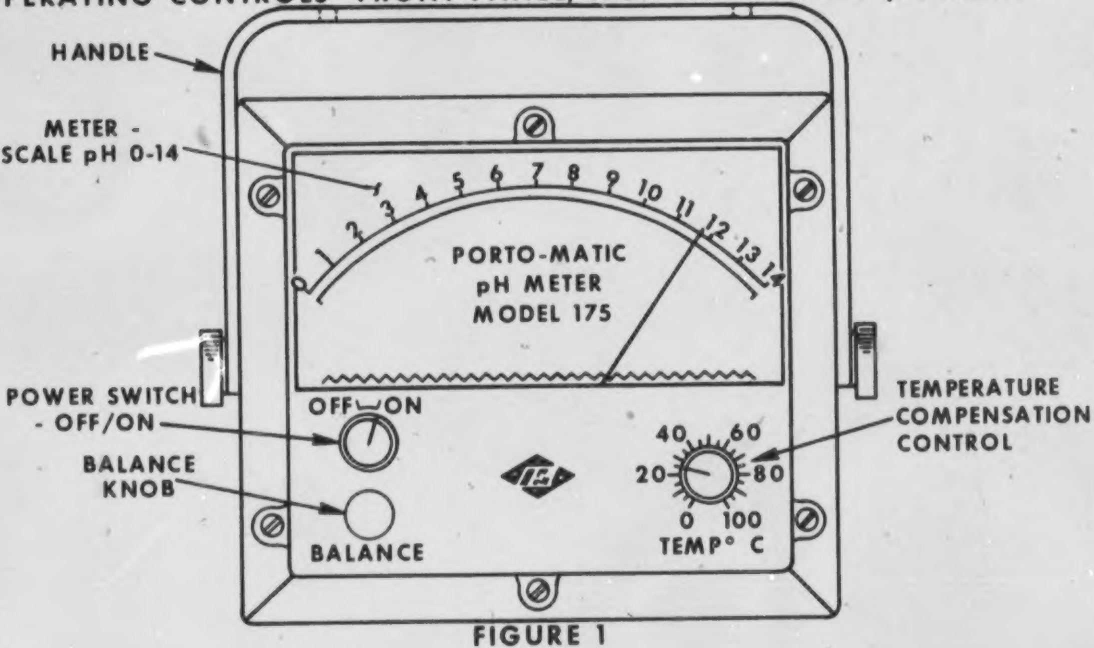
HANDLE
METER - SCALE pH 0-14
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
PORTO-MATIC
pH METER
MODEL 175
POWER SWITCH - OFF/ON
OFF ON
BALANCE KNOB
BALANCE
40
60
20
80
0
100
TEMP° C
TEMPERATURE COMPENSATION CONTROL

FIGURE 1

REAR PANEL, 175 PORTO-MATIC pH METER

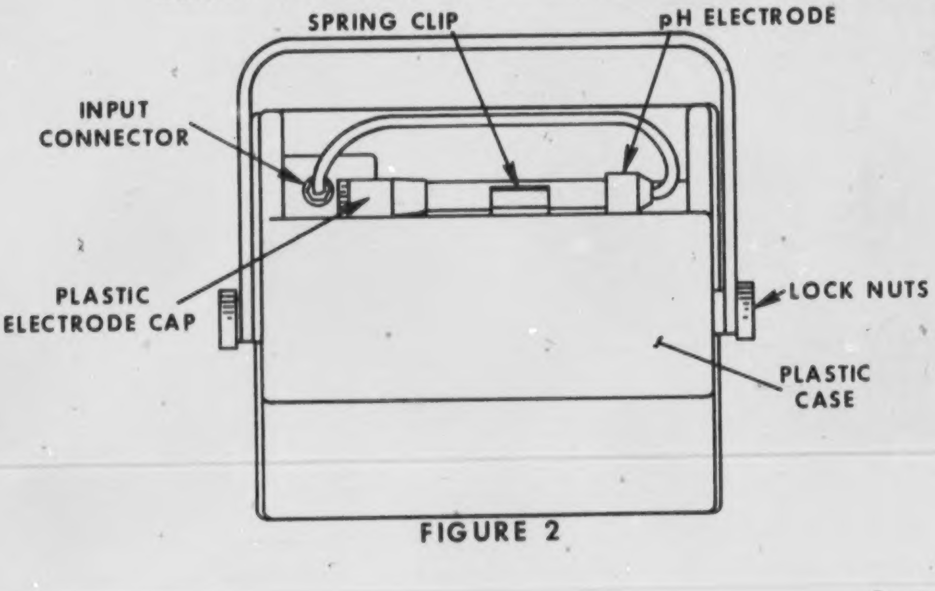
SPRING CLIP
pH ELECTRODE
INPUT CONNECTOR
PLASTIC ELECTRODE CAP
LOCK NUTS
PLASTIC CASE

FIGURE 2

EFFLUENT MONITORING PROCEDURE: pH Determination of Wastewater and Wastewater Treatment Plant Effluents

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Instrument Setup	1. Place pH meter on solid surface.		
	2. Remove electrode from rear panel.	See Fig. 2.	I.A (p. 13)
	3. Twist the cap at the bottom of the electrode to align the filling holes.	See Fig. 2.	
	4. Check the level and saturation of the KCl solution in the reference chamber of the electrode.		
	5. Place the electrode in a 150 ml beaker containing 100 ml of distilled water.		
	6. Turn ON/OFF switch on.		
B. Meter Calibration	1. Set temperature compensation knob to correspond to temperature of buffer solution to be used for calibration.	1a. Previously prepared standard buffer solution pH 6.9 should be used. Buffer solutions can be prepared from the formulas shown in the attached table.	
	2. Transfer 50 ml of pH 6.9 buffer solution into a clean 150 ml beaker.		VII.1 (p. 15)
	3. Turn meter switch "off"	3a. Meter should be "off" when electrode is out of solution.	

EFFLUENT MONITORING PROCEDURE: pH Determination of Wastewater and Wastewater Treatment Plant Effluents

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
	4. Rinse the electrode with buffer solution and immerse it in the beaker containing the pH 6.9 buffer.	4a. Do not allow bubbles to collect around the ceramic junction of the reference chamber.	VII.1 (p. 15)
	5. Turn meter on.		
	6. Adjust the needle on the meter to read 6.9 by turning the balance knob clockwise or counter-clockwise.	6a. Allow adequate time for the glass electrode to come into equilibrium with the sample (approximately 30 seconds).	
	7. Turn the power switch off.		
	8. Repeat calibration with buffer pH 7.4		
	9. Remove the electrode from the buffer and rinse the electrode three times with distilled water.	9a. Use a squeeze type wash bottle.	
	10. Add distilled water to cap.		
	11. Twist the cap on the bottom of the electrode so that the filling holes are closed and water surrounds the glass membrane.	11a. The pH sensitive membrane dehydrates when removed from water. Dry glass electrodes should be soaked in buffer or water for several hours before use.	
	12. Discard buffer solution.	12a. Never pour used buffer solution back into buffer bottle.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Use of Instrument for pH measurement	1. Adjust temperature compensation knob to the temperature of the unknown solution.		
	2. Twist open the electrode cap.		
	3. Immerse the electrode into the unknown.		
	4. Turn the power switch on.		
	5. Allow adequate time for the glass electrode to come into equilibrium with the sample (approximately 30 seconds).	5a. Do not allow bubbles to collect around the ceramic junction of the reference chamber.	
	6. Determine pH of unknown solution by observation of meter needle on pH scale of instrument.	6a. Swirl probe several times before taking reading. 6b. Take reading with mirror reflection of needle obscured by needle.	
	7. Enter the result on the appropriate report form. Record your value to the nearest 0.1 pH unit.		
	8. Turn off instrument.		
	9. Rinse the electrode with distilled water.		
	10. Add water or buffer to cap prior to closing to prevent dehydration of electrode.		
	11. Close the cap of the electrode.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Maintenance	1. Check the level and saturation of potassium chloride in the reference chamber of electrode.	1a. The reference chamber of the pH electrode system should always be kept nearly full with saturated potassium chloride solution.	VII.D (p. 15)
	2. Keep the glass membrane wet with distilled water when not being used.		
	3. Do not contaminate standard buffer solution.		
	4. Turn the instrument off when not in use.	4a. The pH meter is a battery-operated instrument.	
E. Trouble Shooting	1. Erratic needle movement:	1a. Erratic needle movement:	
	a. Fill the measuring chamber completely	Can be caused by bubbles around the ceramic reference junction.	
	b. Soak the external surface of the ceramic plug in warm water.	Can be caused by contamination or salt crystallization of the reference ceramic plug.	
	c. Resaturate the reference chamber.	Can be caused by unsaturation of the reference chamber.	
	2. No instrumental response when measurement is taken.	2a. Exchange electrode or electrodes with new electrodes. 2b. If porous plug of electrode is clogged, take appropriate action to clean. See electrode specification sheet.	

EFFLUENT MONITORING PROCEDURE: pH Determination of Wastewater and Wastewater Treatment Plant Effluents

TRAINING GUIDE

SECTION	TOPIC
I*	Introduction
II	Educational Concepts - Mathematics
III	Educational Concepts - Science
IV	Educational Concepts - Communications
V	Field and Laboratory Equipment
VI	Field and Laboratory Reagents
VII*	Field and Laboratory Analysis
VIII	Safety
IX	Records and Reports

*Training guide materials are presented here under the heading marked *. These standardized headings are used throughout this series of procedures.

TRAINING GUIDE NOTE	REFERENCES/RESOURCES

I Introduction

1. pH General Considerations

pH is a term used to describe the intensity of the acid or alkaline condition of a solution. The concept of pH evolved from a series of developments that led to a fuller understanding of acids and alkaline solutions (bases). Acids and bases were originally distinguished by their difference in physical characteristics (acids-sour, bases-soapy feel). In the 18th century it was recognized that acids have a sour taste (vinegar-acetic acid), that they react with limestone with the liberation of a gaseous substance (carbon dioxide) and that neutral substances result from their interaction with alkaline solutions.

Nebergall, W. H., Schmidt, F. C. and Holtzclaw, Jr., HF., College Chem., 2nd Ed. Heath & Co., Boston, 1963

Acids are also described as compounds that yield hydrogen ions when dissolved in water. And that bases yield hydroxide ions when dissolved in water. The process of neutralization is then considered to be the union of hydrogen (H^+) ions and hydroxyl (OH^-) ions to form neutral water ($H^+ + OH \longrightarrow H_2O$).

It has been determined that there are 1/10,000,000 grams of hydrogen ions and 17/10,000,000 grams of hydroxyl ions in one liter of pure water. The product of the H^+ and OH^- ions equal a constant value. Therefore, if the concentration of the H^+ ions is increased there is a corresponding decrease in OH^- ions. The acidity or alkalinity, hydrogen ion concentration of a solution is given in terms of pH. The pH scale extends from 0 to 14 with the neutral point at 7.0.

TRAINING GUIDE NOTE	REFERENCES/RESOURCES
2. Electrode Design About 1925 it was discovered that an electrode could be constructed of glass which would develop a potential related to the hydrogen-ion concentration without interference from most other ions. The glass pH electrode is the nearest approach to a universal pH indicator known at present. It works on the principle of establishing a potential across a pH sensitive, glass membrane whose magnitude is proportional to the difference in pH of the solution separated by this membrane. All glass pH indicating electrodes have a similar basic design. Contained on one side of an appropriate glass membrane is a solution of constant pH. In contact with the other side of this pH sensitive glass is the solution of unknown pH. Between the surfaces of the glass membrane, a potential is established which is proportional to the pH difference of these solutions. As the pH of one solution is constant, this developed potential is a measure of the pH of the other. To measure this potential, a half-cell is introduced into both the constant, internal solution and into the unknown, external solution. These half-cells are in turn connected to your pH meter. The internal reversible half-cell sealed within the chamber of constant pH is almost exclusively a wire of silver-silver chloride. The external reversible half-cell is often silver-silver chloride. If both the internal and external electrodes are combined in a common pH measuring device, the electrode is a combination pH electrode.	Sawyer, C.N., and McCarty, P.L. Chem. for San. Eng. 2nd Ed. McGraw-Hill, NY, 1967 Instruction-Manual IL 175 PORTO-matic pH meter Instrumentation Laboratory, Inc. Lexington, Mass.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
	As the function of these half-cells is to provide a steady reference voltage against which voltage changes at the glass pH sensitive membrane can be referred, they must be protected from contamination and dilution by the unknown solutions. This is accomplished by permanently sealing the internal half-cell in a separate chamber which makes electrical contact to the unknown solution through a porous ceramic plug. This ceramic plug allows current to flow, but does not permit exchange of solution to this chamber. Gradually the KCl solution is slowly lost, therefore a filling port is placed in this electrode so that additional saturated potassium chloride can be added.	
VII	Laboratory Analysis 1. Instrument Calibration The pH balance control, by adding a voltage in series with the pH electrode system, allows the operator to adjust the meter readout to conform to the pH of the calibrating buffer. In general, calibrate the meter in the general range of the unknown solutions. Appropriate buffers can be selected (pH 4.0, 6.8, 7.4 and 10.0). Always set the temperature compensator on the instrument to the temperature of the standard buffer solution. For most accurate analysis the pH of the sample should be determined, and then buffered solutions of a pH above and below the determined pH should be selected to re-calibrate the instrument and the determination of the pH of the sample repeated for a final reading.	
VII	Maintenance Practices 1. The reference chamber of the pH electrode system should always be kept nearly full of saturated KCl solution. Routinely check the level and saturation	

TRAINING GUIDE NOTE	REFERENCES/RESOURCES
of potassium chloride in this reference chamber and add saturated KCl if necessary. 2. The pH sensitive glass membrane dehydrates when removed from water, and thus it is imperative that dry electrodes be soaked in buffer or water for several hours before use. To avoid this break-in period always keep the glass pH sensitive membrane wet between periods of use. 3. The buffers are pH standards; do not contaminate them. 4. The meter is a battery operated instrument. To conserve the battery life, turn the instrument off when not in use.	

Table 144(1): Preparation of pH Standard Solutions

Standard Solution (Molality)	pH at 25 C°	Weight of Chemicals Needed per 1,000 ml of Aqueous Solution at 25 C°
Primary standards		
Potassium hydrogen tartrate (saturated at 25 C)	3.557	6.4g$KHC_4H_4O_6$*
0.05 potassium dihydrogen citrate	3.776	11.41g$KH_2C_6H_5O_7$
0.05 potassium hydrogen phthalate	4.008	10.12g$KHC_8H_4O_4$
0.025 potassium dihydrogen phosphate + 0.025 disodium hydrogen phosphate	6.865	3.388gKH_2PO_4† + 3.533gNa_2HPO_4†‡
0.008695 potassium dihydrogen phosphate + 0.03043 disodium hydrogen phosphate	7.413	1.179gKH_2PO_4† + 4.302gNa_2HPO_4†‡
0.01 sodium borate decahydrate (borax)	9.180	3.80g$Na_2B_4O_7 \cdot 10H_2O$‡
0.025 sodium bicarbonate + 0.025 sodium carbonate	10.012	2.092g$NaHCO_3$ + 2.640gNa_2CO_3
Secondary standards		
0.05 potassium tetroxalate dihydrate	1.679	12.61g$KH_3C_4O_8 \cdot 2H_2O$
Calcium hydroxide (saturated at 25 C°)	12.454	1.5g$Ca(OH)_2$*

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*Approximate solubility
†Dry chemical at 110-130 C° for 2 hr.
‡Prepare with freshly boiled and cooled distilled water (carbon dioxide-free)

NAME ____________________

LABORATORY RESULTS

SAMPLE	pH RESULT
Sample 1	
Sample 2	
Sample 3	
Buffer 4	
Buffer 9	

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

COLLECTION AND HANDLING OF BACTERIOLOGICAL SAMPLES FROM A WASTEWATER TREATMENT FACILITY

as applied in

WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

Developed by the

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

EFFLUENT MONITORING PROCEDURE: Collection and Handling of Bacteriological Samples

This Procedure was developed by:

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EFFLUENT MONITORING PROCEDURE: Collection and Handling of Bacteriological Samples

1. Objective:

Proper technique for the collection and handling of a sample for bacteriological examination taken from a wastewater treatment facility.

2. Brief Description of Analysis:

After assembly of necessary equipment and travel to the sample site, the sample is collected in a manner which does not bias the sample. Samples are collected in a suitable, labeled, sterilized sample bottle which may contain chemical agents to inactivate chlorine disinfectant or reduce effects of toxic metals if these are present in the sample.

Bottle handling and filling, using hand or suitable holding device, is accomplished in a manner to avoid entry of non-representative contamination which can alter the bacteriological test results.

Cooling or refrigeration, if the sample is held for longer than one hour before bacteriological laboratory testing, is accomplished at less than 10°C but avoiding freezing of the sample. Holding or transporting in this condition can be for no more than 6 hours before the bacteriological testing must be initiated for which another 2 hours is allowed as processing time.

3. Applicability of this Procedure:

Treatment of Interferences in Samples:

This procedure includes directions for dechlorination of samples sufficient to act upon samples containing up to 15 mg of residual chlorine per liter. Also, directions are given for metal detoxification by cnelation if these materials are present or expected in the sample.

Source of Procedure: Standard Methods for the Examination of Water and Wastewater, 14th ed., 1975.

EFFLUENT MONITORING PROCEDURE: Collection and Handling of Bacteriological Samples

Laboratory Equipment and Supplies

General Description of Equipment Used in the Process

Sample Bottle

Bacteriologically inert; resistant to sterilizing conditions; capacity at least 100 ml plus air space; containing dechlorinating agent if a sample containing chlorine is anticipated; and containing a chelating agent if metals are anticipated in sample.

Label

Clean and unused; non-smearing if wetted; sufficient size for needed information; can be attached securely to sample bottle.

Marking Device

Non-smearing if wetted; permanent marking.

Sampler Device

Unnecessary if bottle can be hand dipped; line, wire, etc., if distance to sample water is short; special apparatus if distance to sample water is sufficient to make line unwieldy or if sample water is reached with difficulty as through manholes, ports, etc.

Germicide and Sponge

Disinfecting agent.

Rubber Gloves

Undamaged condition and of proper size.

Container

Ice chest with cover.

Reagents

EDTA (Ethylene dinitrilotetra acetic acid)
Sodium thiosulfate ($Na_2S_2O_3.5H_2O$)

EFFLUENT MONITORING PROCEDURE: Collection and Handling of Bacteriological Samples

Capital Equipment

Autoclave

Providing uniform temperatures up to, and including 121°C, equipped with an accurate thermometer, pressure gauges, safety features, saturated steam power lines and capable of reaching required temperature within 30 minutes.

Alternately

A pressure cooker can be substituted if:

* effluent pressure gauge
* thermometer bulb 2.5 cm above water level

Balance

0.1 g sensitivity at load of 160 g.

Oven

Drying and sterilizing. Capable of uniform temperatures and with suitable thermometer to register accurately in the range 160 - 180°C.

Refrigerator (at laboratory)

Set for 2°-10°C.

Distillation Apparatus, Water

In order of preference, the systems are of stainless steel, quartz, vycor and pyrex glass. Tin-lined hardware is acceptable but because of maintenance problems is best avoided in preference to the above. Plumbing should be of stainless steel, pyrex or plastic PVC material. Storage reservoirs of stainless steel and dust protected. Produced water must be of suitable bacteriologic quality (test described in Standard Methods, 14th Edition, P. 887.)

Alternately

Distilled water meeting this quality criteria can be purchanged, eliminating the need for the distillation apparatus.

Washer, Glassware

Operate at 180°C during hot detergent cycle; hot rinse cycle of 6 to 12 successive washings; and final rinse of bacteriologically suitable distilled or deionized water.

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTE
A. Presampling Procedures			
1. Sample bottles	1. Check sample bottles for acceptability	1a. Suitable material of at least 100 ml capacity. 1b. Bottles should not be chipped, cracked or otherwise damaged. No deposits or extensive glass scratches or etched surfaces can be tolerated. 1c. Bottle covers must not be cracked or otherwise damaged.	V.A.1.1a (p. 17)
2. Labels	1. Check labels for acceptability	1a. Must be clean and unused. 1b. Sufficient quantity for number of samples plus a few extra labels. 1c. Each label must have a means of attachment to sample bottles. Wire or cord is desirable and such attachments as scotch tape, electrical tape, etc. must be avoided as these are affected by water immersion. 1d. Labels can vary from that which is completely blank to a type which is required by the facility, agency, authority, etc.	
3. Label Marker	1. Check label marker for acceptability	1a. Marker must be of a non-smearing permanent type. 1b. Marker is operable.	
4. Sampling Device	1. Check sampling service for condition	1a. A number of suitable sampling devices are available and the function to a) provide weight to allow the sampling device to reach a depth without drifting; b) provide an anchoring point for the sterile bottle or chamber; c) have a tripping mechanism to allow entry of sample to the collector; and d) provide a means of lowering the device to depth and returned to surface. Check operation of each of these areas. 1b. Some types of samplers do not utilize a bottle but may function with a bulb, bladder, etc. It will be necessary for the sampler to acquaint	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Presampling Procedures (Continued)		himself to the specific device being utilized at his facility.	
5. Germicide	1. Availability of germicide	1a. Provides a means of disinfecting any spillage of sewage or sample. 1b. Must not be used in a manner where it could find its way to contaminate sample, equipment, etc. 1c. Sufficient quantity available (normally about one pint will suffice for any contingency).	VI.A.5 (p. 18)
6. Rubber Gloves	1. Check rubber gloves for acceptability	1a. Proper sized to fit comfortably. 1b. Must not be punctured.	
7. Chest, insulated	1. Check chest for acceptability	1a. Must be of sufficient size to accommodate samples to be taken. 1b. Must be undamaged so that cold temperature will be retained inside chest. Must have tight fitting cover. 1c. Contains ice to quickly chill samples. Must not have too much water volume since this can compromise sample.	
8. Refrigerator	1. Check refrigerator for acceptability	1a. Sufficient shelf space for samples. Use of refrigerator will only be necessary if it is not possible to run samples immediately upon return to laboratory. 1b. Temperature setting 2^{o}-10^{o}C.	
9. Glassware	1. Wash all glassware in hot detergent solution	1a. Nontoxic detergent 1b. Be sure all contents and markings are washed away	V.A.9.1-4 (p. 17)
	2. Rinse in hot tap water	2a. At least 6-12 successive rinses to remove detergent residue	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Presampling Procedures (Continued)			
	3. Rinse in distilled water or deionized water	3a. Water must be known to be suitable for bacteriological operations. 3b. 6 to 12 successive rinses recommended to remove all residues.	VI.A.9.3a (p. 18)
	4. Dry in air	4a. No visible spots or scum; glass should be clean and sparkling. 4b. Glassware must not contain bacteriostatic or inhibitory residues.	V.A.9.4b (p. 17)
		IMPORTANT The following special conditions may apply to the sample to be analyzed: * If sample is chlorinated effluent which contains copper, zinc, or heavy metals, do operating procedures A.10, A.11, and A.12 completely. * If sample is unchlorinated effluent which contains copper, zinc, or heavy metals, eliminate steps: A.10, and A.12.1. * If the sample is chlorinated effluent which does not contain copper, zinc, or heavy metals, eliminate steps: A.11 and A.12.2. * If the sample is unchlorinated and contains no copper, zinc, or heavy metals, eliminate steps: A.10, A.11, A.12.1 and A.12.2.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Presampling Procedures (Continued)			
10. Sodium thiosulfate solution	1. Weigh 10.0 grams of sodium thiosulfate	1a. Used for dechlorination of samples. 1b. Use of trip balance accepted.	
	2. Dissolve in 50-60 ml distilled water	2a. 100 ml graduated cylinder satisfactory.	
	3. Add distilled water to bring final volume to 100 ml.		
	4. Transfer to labeled bottle	4a. Should be labeled as 10% sodium thiosulfate and stored in refrigerator.	
11. Ethylene-dinitrilotetra acetic acid (EDTA) solution	1. Weigh 15.0 grams of EDTA	1a. Used for water samples high in copper or zinc, or wastewater high in heavy metals. 1b. Use of trip balance accepted.	
	2. Dissolve in 50-60 ml of distilled water	2a. 100 ml graduated cylinder satisfactory.	
	3. Add distilled water to bring final volume to 100 ml		
	4. Transfer to clean labeled bottle	4a. Labeled as 15% Ethylene dinitrilotetra acetic acid (EDTA) and stored in refrigerator.	
12. Sample bottle preparation	1. Deliver 0.1 ml or 0.2 ml thiosulfate solution to each sample bottle. (.1 ml to 4 oz. or 120 ml size and .2 ml to 6-8 oz. or 250 ml size)	1a. Use 1 ml pipet. 1b. Provides adequate sodium thiosulfate for neutralizing chlorine in sample.	V.A.12.1-6

EFFLUENT MONITORING PROCEDURE: Collection and Handling of Bacteriological Samples

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Presampling Procedures (Continued)			
	2. Deliver .3 ml or .6 ml of 15% EDTA solution to each sample bottle (.3 ml to 4 oz. or 120 ml. size and .6 ml. to 6-8 oz. or 250 ml size	2a. Use 1 ml pipet. 2b. Provides adequate EDTA chelating agent for metals in sample 2c. Return stock solution of EDTA to refrigerator.	
	3. Place cover on sample bottle		
	4. Place paper or metal foil cover over bottle cap or stopper	4a. Protects opening of sample bottle from accidental contamination.	
	5. Sterilize sample bottles	5a. 1 hour at 170°C.	
	6. Store sample bottles in clean, dry place until used		
B. Travel: Assembly Point to Sample Point			
1. Initial Sampling	1. Proceed to initial sample point	1a. Transport equipment with care. 1b. Upon arrival recheck as to correctness of designated sampling point.	
	2. Prepare sample station for collection of sample	2a. Remove manholes, ports, access panels, etc., if necessary. 2b. Note safety hazards at site. It is necessary to have a partner if potentially hazardous conditions can result in injury or death if another person is not available for help.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Sample Collection			
1. Spigot or tap	1. Put on rubber gloves		
	2. Flush spigot	2a. Must have direct main connection. 2b. Full flow flush for 2-3 minutes or enough to clear service line.	
	3. Remove hood and cap from sample bottle	3a. Remove as unit. 3b. Discard slip of paper which is between cap and bottle. 3c. Protect unit from contamination. Usual method is to hold cap in left hand (if right-handed) and have bottle in right hand.	
	4. Let sample run into bottle	4a. No rinsing of bottle. Especially important if bottle contains sodium thiosulfate or EDTA. 4b. Fill about 3/4 full so that a mixing space is available for thorough sample mixing prior to laboratory operations.	
	5. Replace cap and hood on bottle	5a. Secure closure but not excessively tightened or wedged on bottle.	
	6. Label bottle	6a. Fill all items required by local authorities.	
	7. Place bottle on ice in ice chest	7a. Do not immerse bottle in water. Remove excessive water if present in chest. 7b. Cover chest.	
2. River, Stream, Lake, holding tank, etc.	1. Put on rubber gloves.	1a. If highly contaminated by direct sewage or count of water is unknown.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Sample Collection (Continued)			
	2. Remove cap and hood from sample bottle	2a. Remove as unit. 2b. Discard slip of paper which is between cap and bottle. 2c. Protect unit from contamination. Usual method is to hold cap in left hand (if right-handed) and have bottle in right hand.	
	3. Hold bottle near base		
	4. Submerge bottle	4a. Note current flow of sample site. Bottle filling operation must be toward flow to avoid contamination from sampler's hand. If current flow is not present, the sampler must push the bottle away from his hand or body to simulate flowing conditions. 4b. Upon entry into water have the neck pointing downward to prevent surface material from entering bottle. 4c. When submerged, tilt bottle neck upward to allow bottle to take in sample water. 4d. If water is shallow the bottle may have to be held in a horizontal position for filling but the same precautions must be observed to avoid contamination. 4e. Allow bottle to fill about 3/4 capacity and then quickly lift out of water. Overfilling or flushing must not occur.	
	5. Replace cap and hood on bottle	5a. Secure closure but avoid excessive tightening or wedging of cap.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Sample Collection (Continued)			
	6. Label bottle	6a. Fill all items required by local authorities.	
	7. Place bottle on ice in ice chest	7a. Do not immerse bottle in water. Remove excessive water if present in chest. 7b. Cover chest.	
3. Device Collected Sample	1. Place sterile sample bottle in device	1a. Some devices may employ sterile plastic bags, containers, etc.	
	2. Immerse sample device to depth required	2a. Mark line to indicate depth measurements.	
	3. Trip device	3a. Allow approximately 10 seconds for bottle to fill or time recommended by manufacturer of device.	
	4. Recover device		
	5. Remove bottle		
	6. Label bottle	6a. Fill all items required by local authorities.	
	7. Place bottle on ice in ice chest	7a. Do not immerse bottle in water. Remove excessive water if present in chest. 7b. Cover chest.	
D. Sample Handling	1. Transport ice chest to laboratory	1a. If sample is bacteriologically processed by the laboratory within 1 hour of collection, icing is not required. 1b. If sample is held for over 1 hour, icing is mandatory.	

EFFLUENT MONITORING PROCEDURE: Collection and Handling of Bacteriological Samples

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Sample Handling (Continued)		1c. Sample delivered to laboratory no later than 6 hours from collection time. 1d. Refrigerator can be used for sample holding or ice chest can be utilized provided that: a. Ice is present for holding time b. Bottles do not become immersed in ice water accumulation c. Chest remains covered	

TRAINING GUIDE

SECTION	TOPIC
I	Introduction
II	Educational Concepts - Mathematics
III	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field & Laboratory Equipment
VI*	Field & Laboratory Reagents
VII	Field & Laboratory Analyses
VIII	Safety
IX	Records and Reports

Training guide materials are presented here under the headings marked. These standardized headings are used through this series of procedures.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.1.1a	Sample bottles must be composed of a material which is nontoxic to bacteria, resistant to solvent action of water, and capable of being sterilized. Preferably an all glass, ground-glass-stoppered closure of about 250 ml size is recommended. Various plastics (polypropylene, Nalgene, etc.) have been found to meet the specifications above, and, closures of the screw cap type are acceptable provided they are, and remain, non toxic to the sample and provide a tight closure.	
A.9.1-4	Information is applicable to both glass or plastic sample bottles having the mandatory qualities mentioned for sampling use.	
A.9.4b	Glassware can be checked for bacteriostatic or inhibitory residues by a bacteriological test procedure which, like the distilled water suitability test, should be undertaken only by professional bacteriologists or in laboratories where this test is done on a regular basis.	Standard Methods for the Examination of Water and Wastewater, 14th ed., 1976, APHA, New York, New York, p. 885.
A.12.1-6	Wide-mouth, glass-stoppered bottles suggested, but other styles accepted. Bottle must be heat stable to sterilizing conditions and not be toxic or nutritive to organisms natural to the sample. If glass-stoppered bottles are used, a strip of paper should be placed in the neck of the bottle before placing the stopper in place in preparation for sterilization. This prevents the glass stopper from "freezing" in place during sterilization. The paper strip is discarded at the time of sample collection.	Standard Methods for the Examination of Water and Wastewater, 14th ed., 1976, APHA, New York, New York, p. 884, p. 904.

A.5 A large number of preparations can be used as germicides varying from dilutions made from purchased concentrates or laboratory preparations made by the worker. A few of the more easily made germicides are as follows:

1. Ethyl Alcohol - 70% solution

   Examples:
   7 ml ethanol + 3 ml distilled water
   70 ml ethanol + 30 ml distilled water
   700 ml ethanol + 300 ml distilled water

2. Isopropyl Alcohol - 70% solution
3. Ethyl Alcohol (70% solution) containing 1% Iodine crystals

   Examples:
   7 ml ethanol + 3 ml distilled water; 0.1 gram Iodine crystals added to the ethanol-water solution

   70 ml ethanol + 30 ml distilled water; 1.0 grams of Iodine crystals added to the ethanol-water solution

4. Isopropyl Alcohol (70% solution) containing 1% Iodine crystals
5. Aqueous KI (potassium iodide) 1% containing 1% Iodine

   Examples:

   1 gram KI + 100 ml distilled water, mix until dissolved, then add 1 gram of iodine crystals and mix until dissolved.

   10 grams KI + 1000 ml (or 1 liter) of distilled water, mix until dissolved, then add 10 grams of Iodine crystals and mix until dissolved.

A.9.3a Distilled water must not contain substances preventing bacterial growth or be highly nutritive. There are required procedures to testing distilled water and should be undertaken only by professional bacteriologists or in laboratories where this is done regularly.

Standard Methods for the Examination of Water and Wastewater, 14th ed., 1976, APHA, New York, New York, p. 881-891.

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

FECAL COLIFORM TEST

by the

MULTIPLE DILUTION TUBE METHOD

as applied in

WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

Developed by the

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

EFFLUENT MONITORING PROCEDURE: Fecal Coliform Test by the Multiple Dilution Tube Method

This Procedure was developed by:

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6 years Research in Sanitary Microbiology

21 years Training of Federal, State and Local personnel in principles and practice of sanitary bacteriology water

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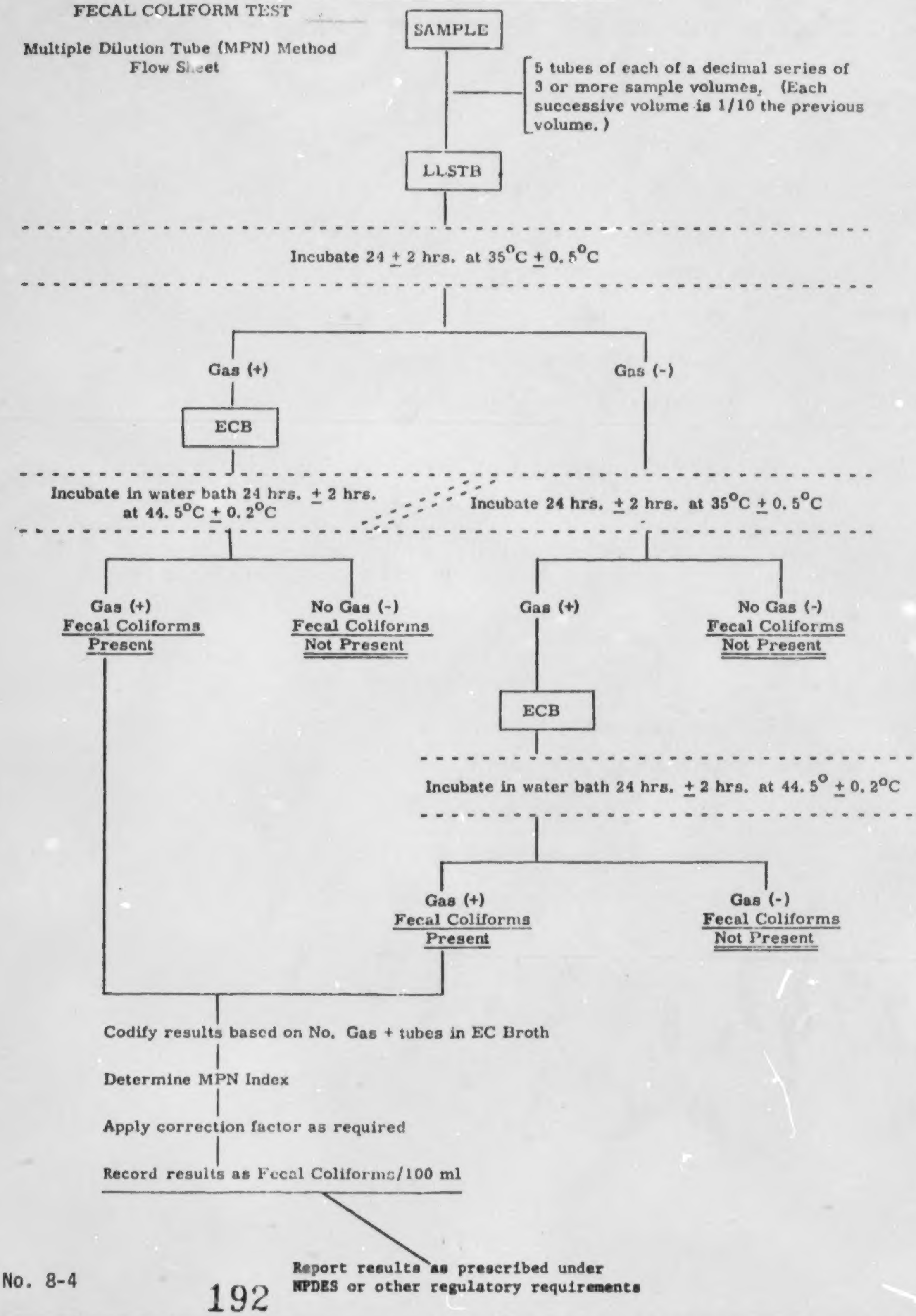
9 years Microbiologist-Instructor

FECAL COLIFORM TEST

Multiple Dilution Tube (MPN) Method
Flow Sheet


SAMPLE
5 tubes of each of a decimal series of 3 or more sample volumes, (Each successive volume is 1/10 the previous volume.)
LLSTB
Incubate 24 ± 2 hrs. at 35°C ± 0.5°C
Gas (+)
Gas (-)
ECB
Incubate in water bath 24 hrs. ± 2 hrs. at 44.5°C ± 0.2°C
Incubate 24 hrs. ± 2 hrs. at 35°C ± 0.5°C
Gas (+) Fecal Coliforms Present
No Gas (-) Fecal Coliforms Not Present
Gas (+)
No Gas (-) Fecal Coliforms Not Present
ECB
Incubate in water bath 24 hrs. ± 2 hrs. at 44.5° ± 0.2°C
Gas (+) Fecal Coliforms Present
Gas (-) Fecal Coliforms Not Present
Codify results based on No. Gas + tubes in EC Broth
Determine MPN Index
Apply correction factor as required
Record results as Fecal Coliforms/100 ml
Report results as prescribed under NPDES or other regulatory requirements

EFFLUENT MONITORING PROCEDURE: Fecal Coliform Test by the Multiple Dilution Tube Method

1. In wastewater effluent quality control, the objective of the test may be one or both of the following:

   a. To determine whether the bacteriological quality of the effluent meets quality requirements set by law or by regulatory authority.

   b. To determine overall efficiency of the treatment process in reducing the bacterial content of the wastewater being treated.

2. Brief Description of Analysis:

   Three or more decimal series dilutions of a sample (for example: five fermentation tubes with 10-ml portions, another five tubes with 1-ml portions, etc.) are inoculated into lactose lauryl sulfate trytose broth (LST) and incubated at $35^{\circ}C \pm 0.5^{\circ}C$. After 24 hours and again after 48 hours the LST cultures are examined and results recorded for gas production. Cultures showing gas are transferred at each examination time to EC Broth (EC) fermentation tubes and incubated at $44.5^{\circ} \pm 0.2^{\circ}C$ in a water bath. EC cultures are examined for evidence of gas production after 24 hours. At the end of the overall incubation period, results are summarized as positive or negative and coded to represent the number of EC gas-positive tubes for each series. A Table of Most Probable Numbers (MPN) is used with the coded results to determine an MPN Index. This index is corrected (if necessary, since the table is for 5-tube, 10, 1.0, and 0.1 ml series only) to agree with the actual sample volumes indicated. The final result is recorded as Fecal Coliforms per 100 ml of sample.

3. Applicability of this Procedure:

   a. Range of Concentration:

If these dilutions are used	These ranges are covered
1.0; 0.1; 0.01;0.001	20 to 160,000
0.1; 0.01; 0.001; 0.0001	200 to 1,600,000
0.01; 0.001; 0.0001; 0.00001	2,000 to 16,000,000
0.001; 0.0001; 0.00001; 0.000001	20,000 to 160,000,000

   b. Pretreatment of Samples

   In accordance with Standard Methods, 14th Ed. (p. 904), and as outlined in EMP, "Collection and Handling of Bacteriological Samples".

This procedure conforms to the fecal coliform test as described in Standard Methods for the Examination of Water and Wastewater, 14th Ed. (1975), p. 922 ff.

EFFLUENT MONITORING PROCEDURE: Fecal Coliform Test by the Multiple Dilution Tube Method

General description of equipment and supplies used in the test analysis

Capital Equipment

Autoclave, providing uniform temperatures up to and including 121°C, equipped with an accurate thermometer, pressure gauges, saturated steam power lines and capable of reaching required temperature within 30 minutes.

Balance, 0.1 g sensitivity at load of 150 g

Air incubator to operate at 35°C ± 0.5°C

Incubator, waterbath, to operate at 44.5° ± 0.2°C and to accommodate tube racks as described separately

Oven, *hot-air sterilizing, to give uniform temperatures and with suitable thermometer to register accurately in range of 160-180°C

pH meter, accurate to at least 0.1 pH unit, with standard pH reference solution(s)

Water distillation apparatus (glass or block tin), or source of distilled water suitable for bacteriological operations.

Reusable Supplies

Apron or coat suitable for laboratory

Baskets, wire for discarded cultures

Bottles, dilution*, 6-oz. screw caps, with 99ml volume level etched on one side

Bottles, sample*, preferred characteristics being 250 ml (6-8 oz.), wide mouth, glass stopper

Bottle, squeeze type, with disinfecting solution

Burner, gas, Bunsen burner type

Cans, pipet, aluminum or steel; not copper (If plastic, or other type of prepackaged disposable pipets are used, this item is unnecessary.)

Metal caps* to fit 20 x 150 mm culture tubes

Pan, to receive discarded contaminated pipets and glassware (must contain disinfectant before use)

Inoculation loop, 3 mm diameter loop of nichrome or platinum-iridium wire, 26 B&S gauge, in holder

Pipets*, 1 ml, with 0.1 ml graduations, Mohr type preferred, sterile, cotton plugged, glass or disposable plastic

Racks, culture type* 10 x 5 openings, to accept tubes at least 20 mm in diameter

Sponge, for cleaning desk top

Tubes, culture*, 150 x 18 mm

Tubes, fermentation*, 75 x 10 mm vials to be inverted in culture tubes

Supplies Used Up in the Analysis (must be replaced when stocks get low)

Distilled water, suitable for bacteriological cultures (note distillation apparatus required in capital equipment)

EC Broth, Dehydrated (recommend purchase of 1/4-lb. units)

Lactose Lauryl Sulfate Tryptose Broth, Dehydrated (recommend purchase of 1-lb. units)

Potassium Dihydrogen Phosphate (KH_2PO_4) (recommend purchase of 1/4 lb. units)

Disinfectant, for bench tops. (Use household bleach solution prepared according to instructions on bottle)

Wax pencils (recommend soft wax equivalent to Blaisdell 169T)

EDTA (ethylene dinitrilotetraacetic acid)

Sodium thiosulfate ($Na_2S_2O_3.5\ H_2O$)

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*Items marked are needed in quantities or require size or space allowances which cannot be specified here, as they vary according to the daily analysis schedule. As a rule-of-thumb, space/size or quantity requirements should be at least 3 times the normal daily requirements. For further information on specifications for equipment and supplies, see the Microbiology Section of the current edition of "Standard Methods for the Examination of Water and Wastewater."

EFFLUENT MONITORING PROCEDURE: Fecal Coliform Test by the Multiple Dilution Tube (MPN) Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pre-Test Procedures		Aa. All pretest procedures completed before starting other first-day procedures.	
1. 35°C incubator set-up, adjustment	1. Place 35°C incubator in permanent location.	1a. Out of drafts or places where it will be in direct sunlight part of day. 1b. Location convenient to laboratory bench. 1c. Convenient source of electric power.	V.A.1.1 (p. 41)
	2. Install thermometer.	2a. Thermometer functions at least in 30°-40°C range and have intervals of 0.5° or less indicated. Meets NBS standards. 2b. Location should be central in incubator. 2c. Mercury bulb thermometer should be fitted with cork or rubber stopper and mounted in small bottle filled with liquid (glycerine, water, or mineral oil).	V.A.1.2 (p. 41)
	3. Install shallow pan of water in bottom of incubator.	3a. In most laboratory incubators a pan having about 1 square foot of area, with water about 1 inch deep, is satisfactory. 3b. Maintains condition of saturated relative humidity, required in bacteriological incubator. 3c. Requires daily check, with addition of water as necessary, to keep water in pan at all times.	V.A.1.3 (p. 41)
	4. Connect incubator to electric power source.	4a. Many incubators have pilot light to indicate power turned on.	
	5. Adjust temperature until stabilized at required temperature.	5a. Manufacturer's instructions for method of temperature adjustment. 5b. Operation must be at 35 ± 0.5°C. 5c. Should allow about 1 hour between adjustments.	V.A.1.5 (p. 41)
	6. Operate bacteriological incubator continuously.	6a. Requires daily check with written temperature record, with adjustment and water addition as necessary.	V.A.1.6 (p. 41)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
2. Water bath incubator setup, adjustment	1. Place water bath incubator in permanent location.	1a. On bench or table surface. 1b. Out of drafts or place in which it will be in direct sunlight part of day. 1c. Location convenient to laboratory bench. 1d. Convenient source of electric power.	
	2. Put water in water bath.	2a. Distilled or deionized water preferred, tap water accepted. 2b. Should be 2-21/2 inches deep above the platform on which the racks of cultures will be placed. Water must be deep enough that when racks of cultures are placed in the water bath the water is as high on the tubes as the top as the culture medium inside the tubes. Yet it must not be so deep as to let the tubes float out of the racks or reach the cap.	
	3. Install thermometer.	3a. Functions at least in 40°-50°C range. Meets NBS standards. Have at least 0.1°C increment markings. 3b. Most water baths provide for corner location for thermometer (for protection from breakage).	
	4. Connect water bath incubator to electric power source and turn on.	4a. Pilot light should come on.	
	5. Adjust temperature until stabilized at required temperature.	5a. Manufacturer's instructions for location and method of temperature adjustment. 5b. Operation must be at 44.5 ± 0.2°C. 5c. Allow about 1 hour between adjustments.	
	6. Operate water bath incubator continuously.	6a. Requires daily check with written temperature record, with adjustment as necessary. 6b. Requires daily check of water level and addition of more as needed. 6c. With tap water in water bath, may require periodic scum removal from inner walls.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
3. Oven, sterilizer, setup	1. Place oven sterilizer in permanent location.	1a. Convenient to source of electric power; usually on table or bench.	V.A.3.1-5 (p. 41)
	2. Install thermometer.	2a. Should indicate the 160°-180°C range, be accurate within this interval, and be marked in 1.0 degree intervals.	
	3. Connect oven sterilizer to power source and turn on.	3a. Usually has pilot light to indicate power on.	
	4. Adjust temperature to stabilize at required temperature.	4a. Operated as near to 170°C as possible; not lower than 160 nor higher than 180°C .	
	5. Operate oven sterilizer only when needed. Turn off when not in use.	5a. Turned ON in advance of need to permit reaching required temperature before introducing material to be sterilized. 5b. Oven sterilizer used to sterilize dry glassware, metal objects. 5c. Oven sterilizer not used with culture media, solution, plastics, rubber objects, or with anything containing or including these. 5d. Paper-wrapped glass pipets may be sterilized in oven sterilizer.	
4. Autoclave setup	1. Install and operate autoclave according to manufacturer's instructions.	1a. Autoclaves extremely variable in design and operation; also, potentially dangerous. 1b. Used to sterilize objects made of, or including liquids, rubber, culture media. 1c. Glassware may be autoclave sterilized but must be dried afterward. 1d. Most plastics not sterilized in autoclave; plastics usually require chemical sterilizers. 1e. Autoclave usually operated at 121°C for 15 min. 1f. Sterilized media must be removed from autoclave as soon as possible after autoclave is reopened.	V.A.4.1 (p. 41)

Dilution Tube (MPN) Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
5. Water distillation equipment	1. Install and operate in accordance with manufacturer's instructions.	1a. Must produce distilled water meeting quality requirements for bacteriological tests.	V.A.5.1-2 (p. 42)
	2. Operate continuously or intermittently as required to maintain adequate supplies of distilled water.	2a. Reserve supplies kept in borosilicate glass carboys or in plastic carboys made of material which will not dissolve substances which will affect growth of bacteria. 2b. Same distillation apparatus used for bacteriological purposes may be used for chemical reagents.	
6. pH meter	1. Have unit available and operate in accordance with procedures described in other lab procedures.	1a. Unit for pH check on finished culture media. 1b. Used in preparation of stock solution of potassium dihydrogen phosphate.	V.A.6.1 (p. 42)
7. Glassware	1. Wash all glassware in hot detergent solution;	1a. Nontoxic detergent 1b. Be sure all contents and markings are washed away.	V.A.7.1-4a (p. 42)
	2. Rinse at least once in hot tap water;		
	3. Rinse in distilled water, at least 6 successive times, and,		
	4. Dry in air.	4a. No visible spots or scum; glass should be clean, and sparkling. 4b. Glassware suitable for use in bacteriological operations.	V.A.7.4b (p. 42)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
		The following special conditions may apply to the sample to be analyzed: If the sample is chlorinated effluent which contains copper, zinc, or heavy metals, do operating procedures A.8, A.9 and A.10 completely. If the sample is unchlorinated effluent which contains copper, zinc, or heavy metals, eliminate steps: A.8 and A.10.1. If the sample is chlorinated effluent which does not contain copper, zinc, or heavy metals, eliminate steps: A.9 and A.10.2. If the sample is unchlorinated and contains no copper, zinc, or heavy metals, eliminate steps: A.8, A.9, A.10.1 and A.10.2.	
8. Sodium thiosulfate solution	1. Weigh 10.0 grams of sodium thiosulfate.	1a. Used for dechlorination of samples. 1b. Use of trip balance accepted.	
	2. Dissolve in 50-60 ml distilled water.	2a. 100 ml graduated cylinder satisfactory.	
	3. Add distilled water to bring final volume to 100 ml.		
	4. Transfer to labeled bottle.	4a. Labeled as 10% sodium thiosulfate and stored in refrigerator.	
9. Etylenedinitrilotetraacetic acid (EDTA) solution	1. Weigh 15.0 grams of EDTA.	1a. Used for water samples high in copper or zinc or wastewater samples high in heavy metals. 1b. Use of trip balance accepted.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
	2. Dissolve in 50-60 ml distilled water.	2a. A 100 ml graduated cylinder is satisfactory.	
	3. Add distilled water to bring final volume to 100 ml.		
	4. Transfer to labeled clean bottle.	4a. The bottle should be labeled as 15% Ethylenedinitrilotetraacetic acid (EDTA) and stored in refrigerator.	
10. Sample bottle preparation	1. Deliver 0.1 ml or .2 ml of 10% sodium thiosulfate solution to each sample bottle. (.1 ml to 4 ounce or 120 ml size and .2 ml to 6-8 ounce or 250 ml size)	1a. Use 1 ml pipet. 1b. Provides adequate sodium thiosulfate for neutralizing chlorine in sample. 1c. Return stock sodium thiosulfate solution to refrigerator.	V.A.10.1-6 (p. 42)
	2. Deliver .3 ml or .6 ml of 15% EDTA solution to each sample bottle (.3 ml to 4 ounce or 120 ml size and .6 ml to 6-8 ounce or 250 ml size).	2a. Use 1 ml pipet. 2b. Provides adequate EDTA chelating agent for metals in sample. 2c. Return stock solution of EDTA to refrigerator.	
	3. Place cover on sample bottle.		
	4. Place paper or metal foil cover over bottle cap or stopper.	4a. Protects opening of sample bottle from accidental contamination.	
	5. Sterilize sample bottles in sterilizing oven.	5a. One hour at 170°C. (See A.3)	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
	6. Store sample bottles in clean, dry place until used.		
11. Pipet preparation	1. Inspect pipets to be prepared for use; discard and destroy all having chipped or cracked tips.	1a. Cleanliness of pipet must be equivalent to glassware. 1b. For protection of user when pipetting sample.	V.A.11.1-6 (p. 42)
	2. Insert plug of non-absorbent cotton into mouthpiece of each clean, dry pipet.	2a. Cotton plug must be tight enough to prevent easy removal, either by the pipetting action or by handling, and yet loose enough to permit easy air movement through the plug.	
	3. Place a layer of glass wool or several layers of paper padding in bottom of pipet can.	3a. For protection of pipet delivery tips.	
	4. Place 18-24 pipets in each pipet can, delivery tip down.	4a. Permits removal of sterile pipets from can without contamination by user.	
	5. Sterilize cans of pipets-pipets in oven.	5a. 1 hour at 170°C. (See A.3 of procedures)	
	6. Store cans in clean, dry place until used.	6a. Laboratory cabinet or drawer recommended.	
	7. When can of pipets is opened for first use, pass the exposed ends of the pipets through flame, slowly.	7a. Burns off excess cotton sticking out of pipet mouthpiece. 7b. Cover kept on can at all times except when samples are being inoculated.	V.A.11.7 (p. 43)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
12. Dilution water blanks	1. Prepare stock solution of potassium dihydrogen phosphate (KH_2PO_4); dissolve 34.0 grams of the KH_2PO_4 in 500 ml distilled water. Adjust to pH 7.2 with 1N NaOH, and dilute to 1 liter with distilled water.	1a. Distilled water may be measured in 500 ml graduated cylinder. 1b. Finished solution labeled "Stock KH_2PO_4 for Dilution Water." 1c. Stored in refrigerator. 1d. Discard stock solution and prepare new solution if mold appears.	V.A.12.1.1d (p. 43)
	2. Prepare stock solution of magnesium sulfate ($MgSO_4 \cdot 7H_2O$) by dissolving 50 grams of this chemical in 500-600 mls of distilled water and, after complete dissolving, bring the final volume to 1 liter in a volumetric flask.		
	3. Prepare working solution of dilution water by adding 1.25 ml KH_2PO_4 and 5 ml of the magnesium sulfate stock solution to each liter of distilled water to be made up as dilution water.	3a. 5 ml pipet satisfactory for 1 liter amounts of dilution water. 10 ml pipet better when several liters are being made. 3b. 1-liter graduated cylinder satisfactory for measurement of distilled water. 3c. Use seperate pipets for each solution to prevent contamination.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
	3. Deliver enough working solution to each dilution water bottle so that after sterilization the bottles will contain 99 ± 2 ml of dilution water.	3a. 100 ml graduated cylinder ordinarily satisfactory. Pipetting machine desirable but not mandatory. 3b. Amount cannot be stated exactly, as sterilization evaporation differs from one autoclave to another. Commonly, about 102 ml is required.	V.A.12.3 (p. 43)
	4. Place caps on dilution bottles loosely.		V.A.12.4 (p. 43)
	5. Sterilize in autoclave.	5a. 15 minutes at 121°C. Use "slow-vent" mode of steam evacuation.	
	6. Promptly remove from autoclave, tighten bottle caps, cool to room temperature.		V.A.12.5 (p. 43)
	7. Store in cool place.	7a. Dilution water ready for use. May be stored indefinitely in screw-capped bottles.	V.A.12.7 (p. 43)
13. Preparation of Lactose Lauryl Sulfate Tryptose Fermentation Broth (LLSTB)	1. Weigh 35.6 grams of dehydrated Lactose Lauryl Sulfate Tryptose Broth. Close cover of bottle of dehydrated medium tightly after removal.	1a. Dehydrated media take moisture out of air; can become caked. 1b. Caked media unsatisfactory; should be discarded. 1c. Prepares 100 tubes (enough for 5 tests based on 4 rows of 5 tubes each).	
	2. Dissolve in 1 liter distilled water.	2a. Gentle heat (no boiling) if necessary to complete dissolving medium.	
	3. Place 10 ml of the solution of prepared LLSTB in each culture tube.	3a. Use 150 x 18 mm tubes. 3b. 10 ml pipet, automatic pipetter, or funnel hose and pinchcock assembly are acceptable. 3c. Accuracy of delivery: ± 0.5 ml.	V.A.13.3b (p. 43)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
Preparation of Lactose Lauryl Sulfate Tryptose Fermentation Broth (LLSTB) (Continued)	4. Insert one fermentation vial into each tube of medium, open end down.	4a. Tubes and vials previously washed as indicated A.7.1-4. 4b. Use 75 x 10 mm tubes.	
	5. Place tube cap on each tube culture medium.	5a. After all tubes have been filled and have individual vial.	
	6. Sterilize in autoclave.	6a. Within 1 hour after medium prepared. 6b. Sterilization at 121°C for 15 minutes. 6c. Medium must be removed from autoclave as soon as possible after pressure has returned to normal. Use "slow-vent" mode of steam removal.	
	7. Cool medium to room temperature.	7a. Medium ready for use when cool and individual vials are completely filled with fluid. No bubbles must be present.	
	8. Check pH of finished medium.	8a. Should be pH 6.8 - 7.0.	
	9. If final pH not satisfactory, discard medium and prepare new batch with pH adjustment before sterilization.	9a. pH value ordinarily drops about 0.2 pH unit.	
	10. Store medium in cool dark place.	10a. Not in refrigerator. Usually in laboratory cabinet in darkness. 10b. May be stored up to 1 week if evaporation not more than 10%.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
14. Preparation of EC Broth	1. Weigh 37.0 grams of dehydrated EC Broth. Close cover of bottle of dehydrated medium tightly after removal.	1a. Dehydrated media take moisture out of air, become caked. 1b. Caked media unsatisfactory; discard. 1c. Prepares 100 tubes; this is enough for four to five tests.	
	2. Dissolve in 1 liter distilled water.	2a. Gentle heat if necessary. No boiling.	
	3. Place 10 ml of the solution of prepared EC Broth in each culture tube.	3a. Use 150 x 18 mm tubes. 3b. 10 ml pipet, automatic pipetter, or funnel hose and pinchcock assembly are acceptable. 3c. Accuracy of deliver ± 0.5 ml	
	4. Insert one fermentation vial into each tube of medium, open end down.	4a. Tubes and vials previously washed as indicated in A.7.1-4. 4b. Use 75 x 10 mm tubes.	
	5. Place tube cap on each tube of culture medium.	5a. After all tubes have been filled and vials inserted.	
	6. Sterilize in autoclave.	6a. After all tubes have been capped. 6b. Sterilization at 121°C for 15 minutes. 6c. Medium must be removed from autoclave as soon as possible after pressure has returned to normal.	
	7. Cool medium to room temperature.	7a. Medium ready for use when cool and individual vials are completely filled with fluid. No bubbles must be present.	
	8. Check pH of finished medium.	8a. Should be pH 6.9.	
	9. If out of range 6.8 - 7.0 discard and prepare again with prior adjustment of pH with 1N NaOH or HCl.	9a. Before sterilization most media should be adjusted to 0.2 pH units higher than pH value expected of the sterile medium.	
	10. Store medium in cool dark place.	10a. Not in refrigerator. Usually in laboratory cabinet in darkness. 10b. May be stored up to 1 week if evaporation not more than 10%.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
15. Final equipment and supply check	1. Check to be sure that all equipment and supplies, solutions, and prepared media are ready before starting sample examination.	1a. Check general list of equipment and supplies. 1b. Each test requires (with 4 sample volumes per test) 20 tubes LLSTB; 10-15 tubes EC Broth 1 sample bottle; 1-5 1-ml pipets, sterile; and 1-3 99-ml bottles sterile dilution water	
	2. Make preparations or adjustments as necessary before starting test.		
B. First-day Procedures			
1. Equipment Maintenance	1. Check, record, and adjust incubator temperature.	1a. See A.1.1-6.	
	2. Add water to pan in incubator as necessary		
2. Sample collection	1. Collect sample.	1a. Locations as selected by plant management. 1b. Sampling methods as described in procedure "Sample Collection and Handling for Bacteriological Tests" or in Standard Methods.	
	2. Record sampling information	2a. Most plants have sample tag of some type which includes such information as date, time, place of sampling, name of sample collector, and other information as may be required.	
	3. Transport sample to laboratory	3a. Taken to laboratory without delay. 3b. Samples iced if delay of starting sample test is greater than one hour. No more than 6 hours of transportation time is allowed.	

<table>
<tr><th>OPERATING PROCEDURES</th><th>STEP SEQUENCE</th><th>INFORMATION/OPERATING GOALS/SPECIFICATIONS</th><th>TRAINING GUIDE NOTES</th></tr>
<tr><td>B. First-day Procedures (Continued)</td><td></td><td></td><td></td></tr>
<tr><td>3. Preparation of laboratory data sheet</td><td>1. Fill in data sheet to show sample information.</td><td>1a. Needed information should be on sample collection tag.</td><td></td></tr>
<tr><td></td><td></td><td>1b. Most data sheets show at least source, date, time of collection, name of sampler, name of analyst, laboratory sample number assigned.</td><td>VII.B.3.1 (p. 44)</td></tr>
<tr><td></td><td>2. Select sample inoculation volumes.</td><td>2a. According to fecal coliform density range predicted for the sample.</td><td>VII.B.3.2 (p. 44)</td></tr>
<tr><td></td><td></td><td>2b. For fecal coliforms per 100 ml in the range
<table>
<tr><th>from</th><th>to</th><th colspan="4">inoculate 5 tubes each of ml</th></tr>
<tr><td>20 -</td><td>160,000</td><td>1.0,</td><td>0.1,</td><td>0.01</td><td>0.001</td></tr>
<tr><td>200 -</td><td>1,600,000</td><td>0.1</td><td>0.01,</td><td>0.001</td><td>0.0001</td></tr>
<tr><td>2,000 -</td><td>16,000,000</td><td>.01</td><td>.001,</td><td>.0001</td><td>.00001</td></tr>
<tr><td>20,000 -</td><td>160,000,000</td><td>.001</td><td>.0001,</td><td>.00001,</td><td>.000001</td></tr>
</table></td><td></td></tr>
<tr><td></td><td></td><td>2c. For chlorinated effluents, 1.0, 0.1, 0.01, and 0.001 ml sample portions are recommended.</td><td></td></tr>
<tr><td></td><td></td><td>2d. For raw (untreated) sewage, use sample portions of 0.0001, 0.00001, 0.000001, and 0.0000001 ml.</td><td></td></tr>
<tr><td></td><td></td><td>2e. For other waters, other combinations of sample volumes may be required, particularly in environmental waters receiving raw or incompletely treated sewage. It may be necessary to conduct exploratory tests.</td><td></td></tr>
<tr><td></td><td>3. Enter information in laboratory data sheet to show sample inoculation volume for each series (row) of 5 tubes.</td><td>3a. Recommend showing sample inoculation volumes in ml or decimal amounts.</td><td></td></tr>
</table>

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. First-day Procedures (Continued)			
4. Lab bench disinfection	1. Disinfect laboratory bench; wipe dry.	1a. Sponge and disinfectant; paper toweling.	
5. Assembly and labeling of culture medium	1. Place 5 tubes of Lactose Lauryl Sulfate Tryptose Broth (LLSTB) in each of 4 rows in culture tube rack. (20 total tubes)	1a. If more than one sample is being tested, rack with 5 x 10 openings can be used to set up two tests.	
	2. Label tubes of culture medium to show sample number, sample volume, and position of tube in the series of 5 tubes per sample volume.	2a. Use labeling code. 2b. Label every tube. Only the experienced worker should take short-cuts in labeling. 2c. Use wax pencil. Soft wax equivalent to Blaisdell 169T is suggested.	VII.B.5.2 (p. 45)
6. Sample inoculation (with dilution as required)	1. Shake sample vigorously.	1a. At least 25 shakes over space of at least 1 foot in 10 seconds or less.	I.B.6.1.1 (p. 34)
	2. Deliver into the labeled LLSTB tubes the sample portions previously selected.	2a. Use sterile 1 ml pipets.	VII.B.6.2-3 (p. 45)

<table>
<tr><th>OPERATING PROCEDURES</th><th>STEP SEQUENCE</th><th>INFORMATION/OPERATING GOALS/SPECIFICATIONS</th><th>TRAINING GUIDE NOTES</th></tr>
<tr><td>B. First-day Procedures (Continued)</td><td></td><td>2b. Table of sample portions
<table>
<tr><th>To get (ml)</th><th></th><th>Deliver (ml)</th><th>From (sample preparations)</th></tr>
<tr><td>1.0</td><td></td><td>1.0</td><td>original sample</td></tr>
<tr><td>0.1</td><td>(1:10)</td><td>0.1</td><td>original sample</td></tr>
<tr><td>0.01</td><td>(1:100)</td><td>1.0</td><td>1:100 dilution</td></tr>
<tr><td>0.001</td><td>(1:1000)</td><td>0.1</td><td>1:100 dilution</td></tr>
<tr><td>0.0001</td><td>(1:10000)</td><td>1.0</td><td>1:10000 dilution</td></tr>
<tr><td>0.00001</td><td>(1:100000)</td><td>0.1</td><td>1:10000 dilution</td></tr>
<tr><td>0.000001</td><td>(1:1000000)</td><td>1.0</td><td>1:1000000 dilution</td></tr>
</table>
2c. Dilutions of original samples
<table>
<tr><th>To get</th><th>Deliver to 99-ml blank</th><th>From</th></tr>
<tr><td>1:100</td><td>1 ml</td><td>original sample</td></tr>
<tr><td>1:10000</td><td>1 ml</td><td>1:100 dilution</td></tr>
<tr><td>1:1000000</td><td>1 ml</td><td>1:10000 dilution</td></tr>
</table>
</td><td></td></tr>
<tr><td></td><td>3. Each time a sample dilution is prepared, shake vigorously, as with the original sample.</td><td>3a. At least 25 shakes over space of at least 1 foot in 10 seconds or less.</td><td></td></tr>
</table>

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
7. Incubation	1. After completion of sample inoculation into LLSTB, shake rack of cultures gently.	1a. Mixes sample with culture medium. 1b. Avoid shaking air into fermentation vials.	
	2. Place rack(s) of cultures in incubator.	2a. 24 hours ± 2 hours at 35 ± 0.5°C	
8. Processing used glassware	1. Drain Sample bottles, dilution bottles, and pipets into sink.	1a. Sterilization unnecessary.	
	2. Wash and dry bottles, pipets.	2a. Meets original cleanliness requirements of glassware. 2b. Glassware ready for reuse.	
9. Lab bench disinfection	1. Disinfect laboratory bench top; wipe dry.	1a. Sponge, disinfectant, paper toweling.	
C. 24-hour Procedures			
1. Equipment maintenance	1. Check, record, and adjust incubator temperature.	1a. See A.1.1-6	
	2. Add water to pan in incubator as necessary.		
2. Disinfection	1. Disinfect laboratory bench top; wipe dry.	1a. See B.4.1	
3. Reading and recording of results.	1. Remove rack(s) of culture(s) from incubator to lab bench.		
	2. Shake culture rack gently.	2a. Hastens release of gas in supersaturated cultures. 2b. [illegible]ust not shake air into fermentation vials.	

EFFLUENT MONITORING PROCEDURE: Fecal Coliform Test by the Multiple Dilution Tube (MPN) Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
	3. Examine each tube for gas production and record results on data sheet.	3a. If present, gas will be trapped in the fermentation vial. 3b. Gas in any quantity is a positive test. 3c. Vials with no gas are a negative test. 3d. Each result appears on line corresponding with the tube label. 3e. All results appear under the "24" of the LLSTB column. 3f. Plus sign (+) means a gas-positive tube. 3g. Minus sign (-) means a gas-negative tube.	III.C.3.3 (p. 39)
4. Transfers	1. Label and assemble tubes of EC Broth.	1a. One tube for each LLSTB gas-positive tube. 1b. Each EC Broth tube label corresponds with label on gas-positive LLSTB tube. 1c. Labeled EC Broth tubes assembled in a culture tube rack in same relative position as gas-positive LLSTB tubes in their rack.	
	2. Transfer each gas-positive tube of LLSTB to a labeled tube of EC Broth.	2a. Label on inoculated tube of EC Broth is the same as the label on the tube of LLSTB from which the transfer is made. 2b. 3-mm inoculation loop. 2c. Loop flame-sterilized before use and between successive transfers. 2d. One loopful per transfer.	VII.C.4.2. III.C.4.2. (p. 40) (p. 47)
	3. Place each inoculated tube of EC Broth in a separate rack, in same relative position as original gas-positive LLSTB tubes in rack.		
	4. After each transfer, place original positive LLSTB tube in discard basket.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
	5. Return negative LLSTB cultures to 35°C incubator.	5a. An additional 24 + 2 hours at 35 + 0.5°C. 5b. Rack should contain all LLSTB gas-negative tubes.	
	6. Place the separate rack of newly inoculated EC Broth tubes in water bath incubator.	6a. 24 ± 2 hours at 44.5 ± 0.2°C. 6b. Must be put in incubator within 30 minutes after transfers have been made.	
5. Processing discarded cultures.	1. Sterilize discarded LLSTB tubes.	1a. Autoclave: 15 minutes at 121°C.	
	2. Remove all labels from culture tubes .	2a. Best done while still warm after autoclave.	
	3. Empty sterilized cultures into sink.		
	4. Wash and dry culture tubes, fermentation vials, and tube caps.	4a. Meets original cleanliness requirements of glassware. 4b. Tubes and caps ready for re-use.	
6. Disinfection	1. Disinfect laboratory bench top; wipe dry.	1a. Sponge and disinfectant; paper toweling.	
D. 48-hour Procedures			
1. Equipment Maintenance	1. Check, record, and adjust incubator temperatures.		
	2. Add water to pan in incubator as necessary.		
2. Disinfection	1. Disinfect lab bench top; wipe dry.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
3. Reading and recording of results	1. Remove rack(s) of culture(s) from incubators to lab bench.		
	2. Shake culture rack(s) gently.		
	3. Examine each tube for gas production and record results on data sheet.	3a. LLSTB tubes will be recorded under the "48" on the LLSTB column.	
4. Transfers	1. Label and assemble tubes of EC Broth.	1a. Corresponding with gas + LLSTB tubes at 48 hours. 1b. One tube for each new LLSTB gas + tube. 1c. Each EC Broth tube label corresponds with label on a gas-positive LLSTB tube. 1d. Labeled EC Broth tubes assembled in a separate culture rack in same relative position as gas-positive LLSTB tubes in rack.	
	2. Transfer each gas-positive tube of LLSTB to a labeled tube of EC Broth.		
	3. Place inoculated tubes of EC Broth in a separate rack.		
	4. After each transfer, place LLSTB tubes in discard basket.		
	5. After all transfers are completed, place all 48-hour gas-negative tubes of LLSTB and all 24-hour tubes of EC Broth in the discard basket.	5a. No further testing of 48-hour gas-negative LLSTB tubes or of any tubes of EC Broth.	
	6. Place newly inoculated EC Broth cultures (if any) in water bath incubator.		

EFFLUENT MONITORING PROCEDURE: Fecal Coliform Test by the Multiple Dilution Tube (MPN) Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
	6a. (alternate) If no cultures remain to be returned to incubator, proceed to "Interpretation of Test Results" and continue as directed.		
5. Processing discarded tubes of media.	1. Sterilize discarded media.		
	2. Remove all labels from culture tubes.		
	3. Empty sterilized cultures into sink.		
	4. Wash and dry culture tubes, fermentation vials, and tube caps.		
6. Disinfection	1. Disinfect laboratory bench top; wipe dry.		
E. 72-hour Procedures			
1. Equipment maintenance	1. Check, record, and adjust incubator temperatures.		
	2. Add water to pan in incubator as necessary.		
2. Disinfection	1. Disinfect lab bench top; wipe dry.		
3. Reading and recording [illegible] results.	1. Remove rack(s) of culture(s) from water bath incubator to lab bench.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. 72-hour Procedures (Continued)			
	2. Shake culture rack(s) gently.		
	3. Examine each tube for gas production and record results on data sheet.		
	4. Place all tubes of EC Broth in discard basket.		
4. Processing discarded tubes of media	1. Sterilize discarded tubes of media.		
	2. Remove all labels from tubes.		
	3. Empty sterilized tubes into sink.		
5. Disinfection	1. Disinfect lab bench top; wipe dry.		
F. Interpretation of test results (Continued)	1. Determine number of EC Broth gas-positive tubes for each group of five tubes of equal sample volumes.	1a. Assume, for instructional purposes, 5 positive 1st row 5 positive 2nd row 2 positive 3rd row 0 positive 4th row	II.F.1 (p. 37) II.F.2 (p. 37)
	2. Write the numbers in the data sheet.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Interpretation of test results (Continued)			

Fecal EC 24 hr.	No. Pos. EC
+	5
+	
+	
+	
+	
+	5
+	
+	
+	
+	
-	2
-	
+	
+	
-	
-	0
-	
-	
-	
-	

6

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Interpretation of test results (Continued)			
	3. Select the 3-digit code which applies to the number of gas-positive tubes of EC Broth.	3a. In a test involving 4 sample volumes this will be based on rows 1, 2, 3, or on rows 2, 3, 4; and 3b. If all tubes are positive in rows 1 and 2, then the 3-digit code is based on rows 2, 3, 4. 3c. In all other cases the 3-digit code is based on rows 1, 2, 3.	II.F.3 (p. 37) (p. 38)
	4. Look up and record on the data sheet the MPN Index.	4a. For the given example the location of the MPN index is shown by the arrow based on the 5-2-0 code.	II.F.4 (p. 32) II.F.5 (p. 38)

Table of Most Probable Numbers (MPN)

No. of Tubes Giving Positive Reaction out of			MPN Index per 100 ml
5 of 10 ml Each	5 of 1 ml Each	5 of 0.1 ml Each	
5	1	0	33
5	1	1	46
5	1	2	63
→ 5	2	0	49 ←
5	2	1	70
5	2	2	94

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Interpretation of test results (Continued)	5. Divide the MPN Index by the number of ml of sample represented by the middle digit of the MPN Code. The number obtained is the MPN (Most Probable Number) per 100 ml of original sample.		
	6. Record the calculated Total Coliforms per 100 ml on the laboratory data sheet.	RESULTS: Fecal Coliform MPN 4900	II.F.6 (p. 38)
G. Reporting of results	1. Report results as prescribed under NPDES or other regulatory requirements.	1a. Report Geometric Mean 1b. See procedure for calculating Geometric Mean described elsewhere in these instructions (EMP units).	

Effluent Monitoring Procedure: Fecal Coliform Test by the Multiple Dilution Tube (MPN) Method

TRAINING GUIDE

SECTION	TOPIC
I*	Introduction
II*	Educational Concepts - Mathematics
III*	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field & Laboratory Equipment
VI*	Field & Laboratory Reagents
VII*	Field & Laboratory Analyses
VIII	Safety
IX	Records and Reports

*Training guide materials are presented here under the headings marked *. These standardized headings are used through this series of procedures.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES

B.6.1.1

These MPN methods for determining bacterial numbers are based on the assumption that the bacteria can be separated from one another (by shaking or other means) resulting in a suspension of individual bacterial cells, uniformly distributed through the original sample when the primary inoculation is made.

Test procedures are based on certain fundamental assumptions:

a. First, even if only one living cell of the test organisms is present in the sample, it will be able to grow when introduced into the primary inoculation medium;

b. Second, growth of the test organism in the culture medium will produce a result which indicates presence of the test organism; and;

c. Third, unwanted organisms will not grow, or if they do grow, they will not limit growth of the test organism; nor will they produce growth effects that will be confused with those of the bacterial group for which the test is designed.

TRAINING GUIDE NOTE

REFERENCES/RESOURCES

Table of Most Probable Numbers (MPN)

No of Tubes Giving Positive Reaction out of			MPN Index per 100 ml
5 of 10 ml Each	5 of 1 ml Each	5 of 0.1 ml Each	
0	0	0	<2
0	0	1	2
0	1	0	2
0	2	0	4
1	0	0	2
1	0	1	4
1	1	0	4
1	1	1	6
1	2	0	6
2	0	0	5
2	0	1	7
2	1	0	7
2	1	1	9
2	2	0	9
2	3	0	12
3	0	0	8
3	0	1	11
3	1	0	11
3	1	1	14
3	2	0	14
3	2	1	17
3	3	0	17
4	0	0	13
4	0	1	17
4	1	0	17
4	1	1	21
4	1	2	26
4	2	0	22
4	2	1	26
4	3	0	27
4	3	1	33
4	4	0	34
5	0	0	23
5	0	1	31
5	0	2	43

Table of Most Probable Numbers (MPN)

No. of Tubes Giving Positive Reaction out of			MPN Index per 100 ml
5 of 10 ml Each	5 of 1 ml Each	5 of 0.1 ml Each	
5	1	0	33
5	1	1	46
5	1	2	63
5	2	0	49
5	2	1	70
5	2	2	94
5	3	0	79
5	3	1	110
5	3	2	140
5	3	3	180
5	4	0	130
5	4	1	170
5	4	2	220
5	4	3	280
5	4	4	350
5	5	0	240
5	5	1	350
5	5	2	540
5	5	3	920
5	5	4	1600
5	5	5	≥2400

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
F.1	For purely qualitative aspects of testing for indicator organisms, it is convenient to consider the tests applied to one sample portion, inoculated into a tube of culture medium, and the follow-up examinations and tests on results of the original inoculation. Results of testing procedures are definite: positive (presence of the organism-group is demonstrated or negative (presence of the organism-group is not demonstrated). The combination of positive and negative results is used in an application of probability mathematics to secure a single MPN value for the sample. To obtain MPN values, the following conditions must be met: a. The testing procedure must result in one or more tubes in which the test organism is demonstrated to be present; and b. The testing procedure must result in one or more tubes in which the test organism is not demonstrated to be present. The MPN value for a given sample is obtained through the use of MPN Tables. It is emphasized that the precision of an individual MPN value is not great when compared with most physical or chemical determinations. Standard practice in water tests made by this organization is to plant five tubes in each of a series of sample increments, in sample volumes decreasing at decimal intervals. As an example, assume that all tubes were positive for a sample portion of 1.0 ml, all five tubes were positive on the portions of 0.1 ml, two of the five 0.01 ml portions were positive, and none of the five 0.001 ml portions were positive.	
F.2	1. The numbers, on the above example, would be 5-5-2-0.	
F.3	1. Pursuing the above example, the code would be 5-2-0.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
F.3 (Continued)	2. Selection of codes is sometimes complicated. For further information study training guide notes and cited references.	Std. Meth. 14-923 ff
F.4	1. Appears on MPN Table (attached to this Section). 2. Pursuing the above example, the MPN Index for MPN Code 5-2-0 would be 49.	
F.5	1. As indicated above, the middle digit is 2; and it represents a sample portion of 0.01 ml. An MPN Index of 49 divided by 0.01 is 4900.	
F.6	The Fecal Coliforms per 100 ml would be recorded as 4900.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
C.3.3	Interpretation of results on LLSTB:	

Development of gas in this medium indicates that the lactose has been fermented. Fermentation of lactose with gas production is a basic characteristic of coliform bacteria. To meet the defination of coliforms, gas must be produced from lactose within 48 hours after being placed in the incubator. If a culture develops gas only after more than 48 hours incubation, then, by definition, it is not a coliform.

Meeting previously discussed assumptions (See B.6.1.1) usually makes it necessary to conduct the tests in a series of stages.

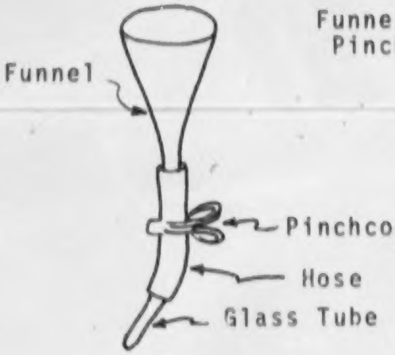
Features of a full, multi-stage test:

a. First stage: The culture medium usually serves primarily as an enrichment medium for the group tested. A good first-stage growth medium should support growth of all the living cells of the group tested, and it should include provision for indicating the presence of the test organism being studied. A first-stage medium may include some component which inhibits growth of extraneous bacteria, but this feature never should be included if it also inhibits growth of any cells of the group for which the test is designed. The Presumptive Test for the coliform group is a good example. The medium supports growth, presumably, of all living cells of the coliform group; the culture container has a fermentation vial for demonstration of gas production resulting from lactose fermentation by coliform bacteria, if present; and sodium lauryl sulfate may be included in one of the approved media for suppression of growth of certain non coliform bacteria. This additive apparently has no adverse effect on growth of members of the coliform group in the concentrations used. If the result of the first-stage test is negative, the study of the culture is terminated, and the result is recorded as a negative test. No further study is made of negative tests. If the result of the first-stage test is positive, the culture may be subjected to further study to verify the findings of the first stage.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
C.4.2	Transfer of gas-positive LLSTB Tubes to EC Broth: This is done in order to find out if the organisms which produced gas from the lactose in LLSTB also can produce gas from a slightly different culture medium (it also contains lactose), and can do so at an elevated temperature ($44.5^{\circ} + 0.2^{\circ}$C.) in a water bath. Practically all coliforms which came from intestinal wastes are able to produce gas from lactose at the elevated temperature of the second medium; and practically all bacteria which produce gas from lactose, but which do not come directly from intestinal wastes, are unable to perform at elevated temperature.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.1.1	Incubator should be kept out of drafts or direct sunlight in order to prevent temperature inside the incubator from changing outside the temperaturn range specified (35° ± 0.5°). Power supply should be selected so that there won't be too many pieces of equipment on the same circuit. Otherwise, circuits will be blown repeatedly.	Standard Methods for the Examination of Water and Wastewater, 14th ed.(1975) APHA, WPCF, AWWA, p. 880 (Hereafter referred to as: Std. Meth. 14: (page no.)
A.1.2	Mercury bulb thermometer usually used in most incubators. Recording thermometer is acceptable, but, it should be calibrated against a mercury bulb thermometer which has been certified by National Bureau of Standards. The NBS certified thermometer always should be used with its certificate and correction chart.	
A.1.3	Saturated relative humidity is required in order to make the incubation more efficient (heat is transferred to cultures faster than in a dry incubator). Furthermore, culture medium may evaporate too fast in a dry incubator.	
A.1.5	Allow enough time after each readjustment to permit the incubator to stabilize before making a new adjustment. At least one hour is suggested.	
A.1.6	Incubator temperature can be held to much closer adjustment if operated continuously. Temperature records should be kept in some form of permanent record. A temperature record book is suggested. If a recording thermometer is used, the charts may be kept as permanent record; if so, be sure that the charts are properly labeled to identify the incubator and the period covered.	
A.3.1-5	Since electric sterilizer will be operated intermittently, care should be taken that it is on a circuit which will not be overloaded when it is turned on.	Std. Meth. 14:881
A.4.1	Autoclaves differ greatly in design and in method of operation. Some are almost like home-style pressure cookers; others are almost fully automatic. This is a subject which requires separate instruction; and should be related to the exact make and model of equipment you will use in your own laboratory.	Std. Meth. 14:881

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.5.1-2	Distilled water in a bacteriological laboratory must not contain substances which will prevent any bacteria from growing in culture medium in which the distilled water is used or will be highly nutritive. There are procedures for testing quality of distilled water; but these should be undertaken only by professional bacteriologists or in laboratories where this is done regularly. Use only glass stills or block tin lined stills.	Std. Meth. 14:645-49 14:888-891 Training Manual (EPA) Current Practices in Water Microbiology
A.6.1	pH Meter: see cited reference	Std. Meth. 14:882
A.7.1-4a	Glassware: See cited reference on pipets and graduated cylinders, media utensils, bottles.	Std. Meth. 14:882-885
A.7.1-4b	Glassware can be checked for bacteriostatic or inhibitory residues by a bacteriological test procedure which, like the distilled water suitability test, should be undertaken only by professional bacteriologists or in laboratories where this test is done on a regular basis.	
A.10.1-6	Sample bottles: Wide-mouthed glass-stoppered bottles suggested, but other styles acceptable. If glass-stoppered bottles are used, a strip of paper should be placed in the neck of the bottle before placing the stopper in place in preparation for sterilization. This prevents the glass stopper from "freezing" in place during sterilization. The paper strip is discarded at the time of sample collection.	Std. Meth. 14:884 14:904
A.11.1-6	Pipets: This procedure is described in terms of reusable glass pipets. However, single-service prepackaged glass or plastic pipets may be purchased and used, if preferred. In case of use of single-service pipets, they will be sterile when purchased, are used one time, and discarded immediately after use. Accordingly, in the step-by-step procedures disregard any instructions about preparation of pipets for reuse in case of using single-service pipets.	Std. Meth. 14:882-883

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.11.7	Passing the opened can of pipets through a flame burns off excess cotton wisps sticking out of the mouthpiece of the pipet. If this is not done, it is almost impossible to control sample measurement accurately.	
A.12.1d.	See cited reference. In time, this solution will become mold-infested. At this time it should be discarded and a new stock solution prepared.	Std. Meth. 14:892
A.12.3	Dilution water preparation: Measurement of dilution water into bottle with a 100 ml graduated cylinder is time-consuming, but effective. An automatic pipetting machine can be considered a luxury, but is a real time-saver.	
A.12.4	If caps are not placed on bottles of dilution water loosely, they may crack in autoclave; furthermore, steam will not be able to get in contact with the material being sterilized. After sterilization, tightening caps on bottles of distilled water will permit them to be kept for long periods.	
A.12.5	Always pack material loosely and away from walls in autoclave when preparing to sterilize. Steam must flow freely around materials being sterilized.	
A.12.7	If water should evaporate noticeably or become contaminated by microbial growth, the bottle of distilled water should be discarded.	
A.13.3b	Funnel, Hose, and Pinchcock Assembly  NOTE: Unit need not be sterile for medium delivery only	

B.3.1 There is no such thing as a "standard" data sheet for bacteriological tests. A simplified data sheet is shown below:

B.3.2 In this procedure, it is recommended that the worker learn to select a series of 4 sample volumes in decreasing amounts as indicated.

It is possible to use as few as three sample volumes, but often the worker will fail to get a measurable result. On the other hand, one could have 5, 6, or even more sample volumes in decreasing amounts.

Fecal Coliform Test
Multiple Dilution Tube (MPN) Method

Sample Type ____________ Lab. No. ________

Station ________ Description ____________

Collection Date ______ Time _____ APM. Temp. _____

Received ________ AM. PM. Examined ________ AM PM.

pH ________ Observations ______________

Amount Sample ml		Presumtive LST		Fecal EC	No. Pos EC				
		24 hr	48 hr	24 hr					

Results:
Fecal coliform MPN

TRAINING GUIDE NOTE | REFERENCES/RESOURCES

B.5.2 Suggested labeling code for tubes:

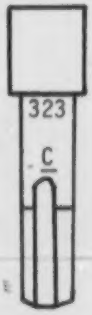
1. Every tube shows the laboratory bench number (323 in example shown on sample data sheet).

2. Below the laboratory bench number on each tube will be found a coded symbol which represents the sample volume and the tube of each series of five. Thus:

Sample volume, ml	Tubes are labeled
1.0	a, b, d, d, e
0.1	$\underline{a}$, $\underline{b}$, $\underline{c}$, $\underline{d}$, $\underline{e}$,
0.01	$\overline{1}a$, $\overline{1}b$, $1\overline{c}$, $\overline{1}d$, 1[illegible]
0.001	2a, 2b, 2c, 2d, 2e
0.0001	3a, 3b, 3c, 3d, 3e
0.00001	4a, 4b, 4c, 4d, 4e
0.000001	5a, 5b, 5c, 5d, 5e
0.0000001	6a, 6b, 6c, 6d, 6e

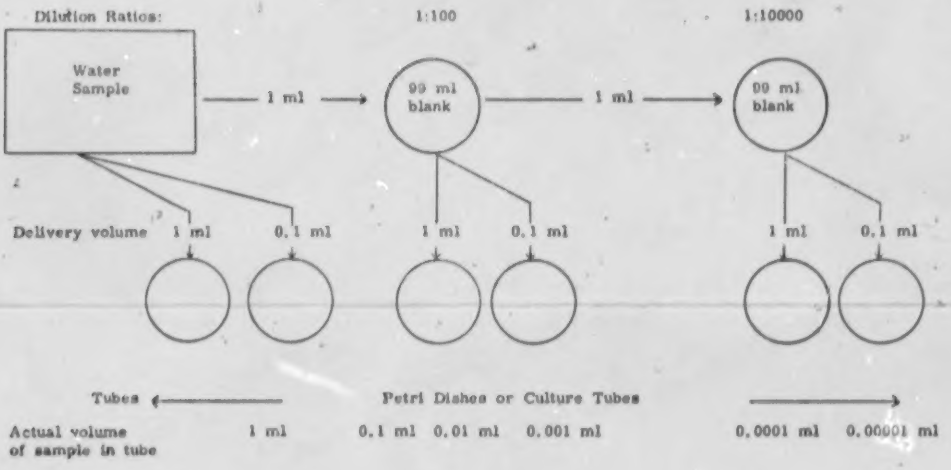
etc., etc.

3. For example, a tube might look something like this, to represent sample No. 323, with the middle tube of a series of five, representing 0.1 ml:



	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
B.6.2-3	Multiple dilution tube tests for quantitative determinations apply a Most Probable Number (MPN) technique. In this procedure one or more measured portions of each of a series of decreasing sample volumes is inoculated into the first-stage culture medium. Through decreasing the sample increments, eventually a volume is reached where only one cell is introduced into some tubes. Each of the several tubes of sample-inoculated first-stage medium is tested independently, according to the principles described. Sample dilutions and inoculations: See Figure 2 as another way to represent sample dilution and inoculation. Note that sample dilutions are made as needed during the inoculation procedure; they are not made up before starting to inoculate tubes of culture medium. Bacteria shall not be suspended in any dilution water for more than 30 minutes at room temperature.	

Figure 2. PREPARATION OF DILUTIONS


Dilution Ratios:
1:100
1:10000
Water Sample
1 ml
99 ml blank
1 ml
99 ml blank
Delivery volume
1 ml
0.1 ml
1 ml
0.1 ml
1 ml
0.1 ml
Tubes
Petri Dishes or Culture Tubes
Actual volume of sample in tube
1 ml
0.1 ml
0.01 ml
0.001 ml
0.0001 ml
0.00001 ml

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
C.4.2	Transfers of LLSTB Transfers can be made, as indicated, with a wire loop having a diameter of at least 3 mm. An alternate method of transfer authorizes the use of an "applicator stick" which is a single service hardwood transfer device. Its dimensions are 0.2 to 0.3 cm in diameter and 2.5 cm longer than the test tube used in the analysis. The term single service denotes that the stick is pre-sterilized and used for a single transfer (LLSTB to EC) and then discarded in the pan containing disinfectant and a new sterile stick used for the next tube to be transferred. Use of this stick technique makes the gas burner unnecessary for the transfer process.	Std. Meth. 14:922

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

FECAL COLIFORM TEST

by the

MEMBRANE FILTER METHOD

as applied in

WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

Developed by the

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

EFFLUENT MONITORING PROCEDURE: Fecal Coliform Test by the Membrane Filter Method

This Procedure was developed by:

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EDUCATION AND TECHNICAL BACKGROUND

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5 years Envorcement Action, Raritan Bay Project, PHS & DI

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EDUCATION AND TECHNICAL BACKGROUND

A.B. - James Millikin University

M.A. - Ohio University

4 years - US Army; Clinical Laboratories; specializing bacteriology

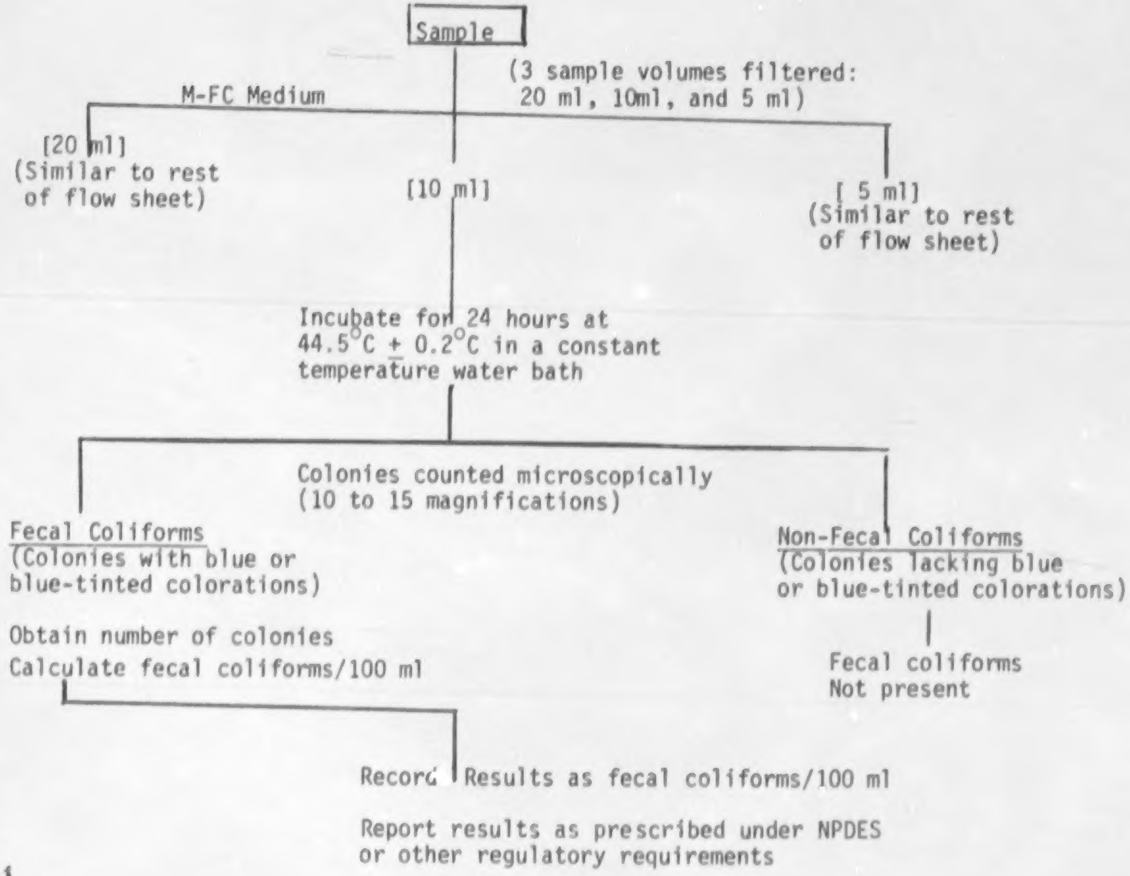
3 years College Instructor - Bacteriology

6 years Research in Sanitary Microbiology

22 years Training of Federal, State and Local personnel in principles and practice of sanitary bacteriology of water

Fecal Coliform Test
Membrane Filter Method

Flow Sheet


Sample
M-FC Medium
(3 sample volumes filtered: 20 ml, 10ml, and 5 ml)
[20 ml] (Similar to rest of flow sheet)
[10 ml]
[5 ml] (Similar to rest of flow sheet)
Incubate for 24 hours at 44.5°C ± 0.2°C in a constant temperature water bath
Colonies counted microscopically (10 to 15 magnifications)
Fecal Coliforms (Colonies with blue or blue-tinted colorations)
Obtain number of colonies
Calculate fecal coliforms/100 ml
Non-Fecal Coliforms (Colonies lacking blue or blue-tinted colorations)
Fecal coliforms Not present
Record Results as fecal coliforms/100 ml
Report results as prescribed under NPDES or other regulatory requirements

EFFLUENT MONITORING PROCEDURE: Membrane Filter Test Method

1. In wastewater effluent quality control, the application of this methodology can be for one or both of the following;

   a. To determine whether the bacteriological quality of the effluent meets quality requirements set by law or by regulatory authority; and,

   b. To determine the bacteriological effects of effluent water on the bacteriological quality of the receiving water.

2. Brief description of analysis:

   A series of measured sample portions is filtered through membrane filters placed individually within a filtering apparatus. Bacteria in the samples are held on the upper surfaces of the filters, while the water passes through and is discarded.

   The membrane filters are placed on a special culture medium, called M-FC Broth, in plastic petri dishes. The inverted petri dishes are placed in a leakproof plastic bag, and incubated totally immersed in a water bath at 44.5° ± 0.2°C for 24 hours ± 2 hours. On M-FC Broth, fecal coliform will grow and develop blue or blue-tinted colonies. Colonies lacking this color characteristic are not considered as fecal coliforms. The blue color may appear only in the centers of the colonies, or entire colonies may be colored. Very few other colonies will develop on the medium at the stated incubation temperature.

   One or two membranes are selected for colony counting on the basis of suitable colony density, and colonies are counted with the aid of a binocular dissecting microscope at a magnification of 10X or 15X. After colonies are counted, a calculation is made in order to report fecal coliforms per 100 ml.

3. Applicability of this Procedure:

   a. Range of Concentration:

      This procedure, as outlined, will detect fecal coliforms within the range of 100 to 1200.

   b. Pretreatment of Samples:

      In accordance with Standard Methods, 14th ed. (p. 904) and as outlined in EMP, "Collection and Handling of Bacteriological Samples."

Analytical Method: Standard Methods for the Examination of Water and Wastewater, 14th ed., 1975, pg. 937 ff.

EFFLUENT MONITORING PROCEDURE: Fecal Coliform Test by the Membrane Filter Method

General description of equipment and supplies used in the test analysis

A. Capital Equipment

Autoclave, steam - providing unifrom temperatures up to and including 121°C and equipped with an accurate thermometer, pressure gauges, saturated steam power lines and capable of reaching required temperatures within 30 minutes.

Balance - Sensitivity of 0.1 gram at a load of 150 grams, with appropriate weights.

Incubator, waterbath - having forced circulation and provided with a cover. Must be capable of providing an incubation temperature of 44.5 ± 0.2°C.

Oven, hot-air- providing unifrom temperatures within the range of 160 - 180°C.

Meter, pH - accurate to within 0.1 pH unit, with suitable standard pH reference solution (s).

Apparatus, water distillation - suitable for bacteriological culture media (alternately, a suitable source is permissible).

Microscope, sterioscopic - 10X - 15X magnification with fluorescent lighting preferred. Alternately, a small fluorescent lamp with magnifier is acceptable.

Refrigerator - Set for less than 10°C but above the freezing temperature. If sample cannot be run within 1 hour refrigeration will be necessary.

Vacuum Source - preferably a pump assembly with suitable hoses and shut-off clamp or valve provided. As an alternate method an aspirator or hand pump with the same provisions are acceptable.

Filtration Unit, MF - a seamless funnel attached to a receptacle bearing a porous plate (screen, porous disc, etc.), stainless steel, glass, porcelain or other suitable material.

B. Reusable Supplies

Apron - suitable for laboratory operations.

Bottle, sample - 250 ml, wide-mouth, glass stopper, with tag (used for sampling operations).

Bottle, squeeze type - containing disinfecting solution.

Burner, gas - suitable for laboratory operations

Can, pipet - non-toxic and sterilizable material (if pre-sterilized disposable-type pipets are used, this item is unnecessary).

Pan, discard - receives contaminated pipets.

Graduate cylinder 100 ml; 500 ml

Pipets, microbiological - 1.0 ml, with 0.1 ml graduations, sterile cotton plugged, glass or disposable types (the disposable types are for one time use and may be glass or plastic).

Pipets, microbiological - 10 ml, with 1 ml graduations, sterile, cotton-plugged, glass or disposable types (the disposable types are for one time use and may be glass or plastic).

Thermometer (water bath) - must indicate within the 40^0 - 50^0C range and have increments of 0.1°C, NBS (National Bureau of Standards) or calibrated against NBS thermometer. Full immersion type preferred.

Thermometer (oven) - must indicate within the 160 - 180^0C range and have increments at least 1.0°C.

Glassware, borosilicate
beaker, 50 ml (for measuring pH, rosolic acid

Flask, volumetric, 1 liter capacity (for stock solution of phosphate buffer)

Flask, Erlenmeyer, 500 ml capacity (for holding buffered distilled rinse water)

Flask, sidearm 1 liter size (for reservoir of MF apparatus. Proper sized and bored rubber stopper is needed to connect MF filtration flask to unit).

Flask, Erlenmeyer, 250 ml (for preparing MFC medium)

Forceps, curved end, round tip

Bottle, small, Methanol or Ethanol volume to cover ends of forceps

Sponge, small, to spread and wipe germicide

Desiccator, media storage. Ideally opaque or darkened and containing desiccating agent to remove moisture.

C. Consumable Supplies:

Dish, petri, disposable, tight fitting plastic, 50 x 12 mm, sterile.

M-FC Broth medium dehydrated, fecal coliform. Distributors Difco, BBL or other equivalent preparation.

Rosolic Acid reagent, 50 gram bottle, Allied Chemical, Olin Matheson Difco or equivalent preparation.

Filter, membrane, 47 mm, 0.45 µm pore size, white, grid marked, sterile.

Pad, absorbent 48 mm, sterile (usually included with membrane packet)

Bag, plastic, water-proof, closure provided or method of sealing bag necessary for water immersion.

Disinfectant, dilute iodine aqueous (water) solution. Commercial preparation or 1 gram iodine crystals and 2 grams potassium iodide to a liter of distilled water.

Methanol or Ethanol, absolute, (for forceps disinfection)

Water, distilled, buffered, sterile (for MF funnel rinsing)

Stock solution, buffer, potassium dihydrogen phosphate

Water, distilled, suitable for bacteriological operations

Potassium Dihydrogen Phosphate (KH_2PO_4) reagent, 1 lb. unit

Data sheet suitable for fecal coliform procedures (has pertinent field information [location, time, sampler, etc.]; lab information [sample, mls filtered, colony counts, etc.]; and effluent monitoring required data [fecal coliforms/100 ml]).

D. Expendable Laboratory Supplies:

Marker, glass or plastic

Glass Wool

Non-absorbent Cotton

Paper, kraft wrapping

Tape, autoclave pressure-resistant

Foil, aluminum, heavy duty

Matches or striker

Toweling, paper

Item needs in quantities or required size or space allowances cannot be specified, as they vary according to the daily analysis schedule. As a rule-of-thumb, space/size or quantity requirements should be at least 3 times the normal daily requirements. For further information on specifications for equipment and supplies, see the Microbiology Section of the current edition of "Standard Methods for the Examination of Water and Wastewater."

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Pretest Procedures		Aa. All pretest procedures completed before starting other first-day procedures	
1. Water bath incubator setup, adjustment (44.5°C ± 0.2°C.)	1. Place water bath incubator in permanent location.	1a. On bench or table surface. 1b. Out of drafts or place in which it will be in direct sunlight part of day. 1c. Location convenient to laboratory bench. 1d. Convenient source of electric power, separate circuit is possible.	V.A.1.1
	2. Put water in water bath.	2a. Distilled or deionized water preferred, tap water accepted. 2b. Should be deep enough to permit total immersion of the plastic bags containing petri dishes. Usually this is about 2½ -3 inches above the platform in the waterbath.	V.A.1.2
	3. Install thermometer.	3a. Functions at least in 40° - 50°C range. Meets NBS standards. Have at least 0.1°C increment markings. 3b. Most water baths provide for corner location for thermometer (for protection from breakage).	V.A.1.3
	4. Connect water bath incubator to electric power source and turn on.	4a. Pilot light should come on.	
	5. Adjust temperature until stabilized at required temperature.	5a. Manufacturer's instructions for location and method of temperature adjustment. 5b. Allow about 1 hour between adjustments. 5c. Operation must be at 44.5 ± 0.2°C.	
	6. Operate water bath incubator continuously.	6a. Requires daily check with written temperature record, with adjustment as necessary. 6b. Requires daily check of water level and addition of more as needed. 6c. With tap water in water bath, may require periodic scum removal from inner walls.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
2. Oven sterilizer setup	1. Place oven sterilizer in permanent position.	1a. A convenient source of electric power.	
	2. Install thermometer	2a. Should read in the 160-180°C range, be accurate within this interval, and be marked in 1.0 degree intervals.	
	3. Connect sterilizer to power source and turn ON.	3a. Pilot light or element heating effect indicates power ON.	
	4. Adjust oven temperature to stabilize at required sterilizing temperature.	4a. 170°C is required temperature.	
	5. Operate when sterilizing is required.	5a. Turned ON in advance of use and checked for temperature stabilization. 5b. Used for dry glassware and metal objects which can be covered by a paper or metallic foil covering. 5c. Not used for culture media, liquids, plastics, and rubber objects or anything containing or including these.	
3. Autoclave setup	1. Install and operate autoclave according to manufacturer's instructions.	1a. Variable in design and operation, and unless properly operated can be dangerous. 1b. Used to sterilize objects made of or including liquids, rubber, and some plastics and for glassware, if desired. 1c. Operated for general sterilization at 121°C (250°F) for a period of 15 minutes after this temperature has been attained. 1d. Sterilized media and liquids must be removed as soon as possible upon sterilization.	V.A.3

EFFLUENT MONITORING PROCEDURE: Fecal Coliform Test by the Membrane Filter Method

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
4. Water distillation equipment	1. Install and operate in accordance with manufacturer's instructions.	1a. Must produce water meeting quality requirements for bacteriological tests.	V.A.4
	2. Operate as required to maintain adequate supplies of distilled water.		
5. pH meter	1. Setup and operate in accordance with manufacturer's recommendations.	1a. Meter must be accurate to at least 0.1 pH unit.	
6. Glassware	1. Cleaned and rinsed using a suitable detergent and hot water.	1a. Non-toxic detergent must be completely removed from glassware.	
	2. Use a final rinse of deionized or distilled water.	2a. 6 to 12 successive rinsings may be required. 2b. Must produce a dry glassware which meets bacteriological requirements for suitability.	V.A.6.2

IMPORTANT

The following Special conditions may apply to the sample to be analyzed:

*If sample is chlorinated effluent which contains copper, zinc, or heavy metals do operating procedures A.7, A.8, and A.9 completely.

* If sample is unchlorinated effluent which contains copper, zinc, or heavy metals, eliminate steps: A.7 and A.9.1

*If the sample is chlorinated effluent which does not contain copper, zinc, or heavy metals, eliminate steps: A.8 and A.9.2

* If the sample is unchlorinated and contains no copper, zinc, or heavy metals, eliminate steps A.7, A.8, A.9.1 and A.9.2.

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
7. Sodium thiosulfate solution	1. Weigh 10.0 grams of sodium thiosulfate.	1a. Used for dechlorination of smaples 1b. Use of trip balance accepted.	
	2. Dissolve in 50-60 ml distilled water.	2a. 100 ml graduated cylinder satisfactory.	
	3. Add distilled water to bring final volume to 100 ml.		
	4. Transfer to labeled bottle	4a. Should be labeled as 10% sodium thiosulfate and stored in refrigerator.	
8. Ethylene-dinitrilotetra-acetic acid (EDTA) solution	1. Weigh 15.0 grams of EDTA.	1a. Used for water samples high in copper or zinc or wastewater high in heavy metals. 1b. Use is trip balance accepted.	
	2. Dissolve in 50-60 ml of distilled water.	2a. 100 ml graduated cylinder satisfactory.	
	3. Add distilled water to bring final volume to 100 ml.		
	4. Transfer to clean labeled bottle.	4a. Labeled as 15% Ethylene-dinitrilotetra-acetic acid (EDTA) and stroed in refrigerator.	
9. Sample bottle preparation	1. Deliver 0.1 ml or 0.2 ml of 10% sodium thiosulfate solution to each sample bottle. (.1ml to 4 oz or 120 ml size and .2 ml to 6-8 oz or 250 ml size)	1a. Use 1 ml pipet 1b. Provides adequate sodium thiosulfate for neutralizing chlorine in sample.	V.A.9.1-6
	2. Deliver .3 ml or .6 ml of 15% EDTA solution to each sample bottle (.3 ml to 4 oz or 120 ml size and .6 ml to 6-8 oz 250 ml size.	2a. Use 1 ml pipet. 2b. Provides adequate EDTA chelating agent for metals in sample. 2c. Return stock solution of EDTA to refrigerator.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
9. Sample bottle preparation (continued)	3. Place cover on sample bottle.		
	4. Place paper or metal foil cover over bottle cap or stopper.	4a. Protects opening of sample bottle from accidental contamination.	
	5. Sterilize sample bottles in sterilizing oven.	5a. 1 hour at 170°C.	
	6. Store sample bottles in clean, dry place until used.		
10. Pipets	1. Insert a plug of non-absorbent cotton into mouthpiece of clean, dry pipet.	1a. Pipets which have chipped or broken tips or tops should be discarded. 1b. Cleanliness of pipet must be equivalent to glassware. 1c. Non-absorbent cotton plug must be tight enough to prevent easy removal, either by the pipeting action or by handling, and yet be loose enough to permit easy air movement through the plug. 1d. Plug protects user from ingesting sample into his mouth.	V.A.10.1-5
	2. Pass plugged end of pipet quickly through burner.	2a. Removes wisps of cotton which prevent fingertip control of pipeting action.	
	3. Insert a layer of glass wool or multi-layer of paper padding in bottom of pipet can.	3a. This protects tips from breakage.	
	4. Place pipet in pipet can with delivery tip downward.	4a. Cotton-plugged end is pipeting end and opposite end is delivery tip.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
10. Pipets (continued)		4b. Approximately 20 1 ml pipets or 12 ml pipets will normally be accommodated in these cans. 4c. Can must be able to withstand steam pressure and dry heat. Toxic materials, such as copper, are not to be used. Aluminum is acceptable.	
	5. Sterilize pipets in oven or autoclave.	5a. At least 1 hour in oven at 170°C, or in autoclave for 15 minutes at 121°C (autoclave set for quick venting of steam). 5b. Cans removed quickly from autoclave with the aid of asbestos gloves. 5c. Cans opened slightly to allow residual steam to escape for a few seconds and then close can.	
	6. Store cans in clean dry place until needed.		
11. Blanks, dilution water	1. Prepare stock solution of potassium dihydrogen phosphate (KH_2PO_4) by dissolving 34.0 grams of this chemical in 500 ml of distilled water and adjusting its pH to 7.2 with 1N NaOH. Dilute to 1 liter in a volumetric flask.	1a. Distilled water may be measured in 500 ml graduated cylinder. 1b. Label to show contents, identity of preparer, and date of preparation. 1c. Stored in refrigerator. 1d. Discarded if mold or turbidity appears.	
	2. Prepare stock solution of magnesium sulfate ($MgSO_4 \cdot 7H_2O$) by dissolving 50 grams of this chemical in 500-600 mls of distilled water and, after complete dissolving, bring the final volume to 1 liter in a volumetric flask.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
	3. Prepare working solution of dilution water by adding 1.25 ml of the potassium dihydrogen phosphate stock solution and 5 ml of the magnesium sulfate stock solution to each liter of distilled water to be used in the preparation of dilution water.	3a. A 10 ml or 5 ml pipet is satisfactory for delivery of both of these stock solutions provided that it has graduation marks to deliver the proper amount. Use separate pipets for each solution to prevent contamination of the stock solutions.	
	4. Deliver enough working solution to each dilution water bottle so that after sterilization the bottle will contain 99 ± 2 ml of dilution water.	4a. Recommended dilution water bottles have a marking at the desired 99 ml quantity. 4b. Amount to be delivered to bottle before sterilization cannot be stated exactly as evaporation is different with differing conditions and autoclaves. Ordinarily about 102 ml will be required.	
	5. Place caps on bottles loosely.		
	6. Sterilize in autoclave.	6a. 15 minutes at $121^{\circ}C$ 6b. Pressure reduced from autoclave gradually. This is usually called "liquid cool" on autoclave dial markings of automatic autoclaves.	
	7. Remove from autoclave tighten bottle caps; cool to room temperature.		
	8. Store in cool place.	8a. Dilution bottles ready for use. May be stored indefinitely. 8b. Some evaporation losses may occur in time and in these cases, sterile similarly prepared water can be added. This is why a calibrated marked bottle is desirable.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
12. Preparation of M-FC medium.	1. Prepare 0.2 normal solution of sodium hydroxide by adding 0.8 grams of solid sodium hydroxide to 100 ml distilled water.	1a. Solution keeps indefinitely, should be protected from evaporation losses with rubber stopper. 1b. CAUTION: sodium hydroxide is corrosive. Add sodium hydroxide to the water, never water to the sodium hydroxide. 1c. Unused solution may be stored until exhausted in regrigerator and labeled as 0.2 N sodium hydroxide.	
	2. Prepare 1% Rosolic Acid solution by dissolving 0.1 gram of Rosolic Acid powder in 10 ml of 0.2 normal solution of sodium hydroxide.	2a. Rosolic acid should be weighed on analytical balance. 2b. Sodium hydroxide solution can be measured with 10 ml pipette. 2c. Unused Rosolic Acid solution can be kept up to 2 weeks if stoppered in refrigerator, and its color remains a dark red; it is best prepared freshly, however.	VIA.12.2
	3. Weigh 3.7 grams of Dehydrated M-FC Broth.	3a. Medium is hygroscopic (picks up moisture from air) and should be stored in tightly stoppered bottle, preferably in the dark, in a desiccator (a closed jar or cabinet which contains materials which take moisture out of the air).	
	4. Place the weighed medium in a clean, dry flask having about 250 ml capacity.	4a. This flask holds more than twice the volume of the required solution because the medium expands and foams when heated and space is required for swirling of flask to mix contents.	
	5. Add 1 ml of the 1% solution of Rosolic Acid to a 100 ml graduate, and fill to the 100 ml mark with distilled water.	5a. Note that this will be a final volume of 100 ml and not 101 ml. 5b. This will be enough for 100 ml of culture medium, or about 50 membrane filter plates. If different amount of medium is required, adjust all materials in proportion.	II.A.12.5

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
	6. Add a small amount of the Rosolic-Acid-distilled water mixture to the flask of weighed powder, and mix until all the powdered medium is dissolved from walls of flask (no sticking powder). Then add the remainder of the water and mix.		
	7. Heat the medium with constant agitation until the boiling point is reached, and then remove from heat and cool promptly to below 45°C.	7a. Agitation necessary to avoid burning the medium. 7b. Cooling is best by holding flask in a stream of cool water.	
	8. Medium is ready for use.	8a. Medium unused on day of preparation may be stored up to 4 days if kept in refrigerator. 8b. Final pH of medium should be 7.4 ± 0.1 units. This pH can be taken by utilizing a small portion of the preparation and discarding after measurement.	
B. First-day procedures 1. Equipment Maintenance	1. Check, record, and adjust incubator temperature, if necessary.	1a. See A.1	
2. Assembly of filtration Material	1. Membrane filtration procedure equipment assembled for analysis.	1a. Funnel clean and sterile 1b. Filtration flask and vacuum system operating 1c. Assembly of: Data sheet, fecal coliform test Sterile petri dishes Sterile membrane filters with absorption pads.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
		Sterile buffered distilled rinse water Forceps and disinfectant container Pencil, marking Sample bottle Sterile pipets, 10 ml Plastic bags Burner, gas Pan, pipet discard with disinfectant	
3. Sample collection	1. Collect sample.	1a. Location selected by plant management. 1b. Sampling method as described in procedure "Sample Collection and Handling for Bacteriological Tests" and in the current edition of Standard Methods.	
	2. Record sampling information.	2a. Most plants have standardized sample tags which includes desired information, such as: Collectors name Date Sample location Time collected Witness Sample delivered to: ________________ Time of delivery 2b. Tag may be retained as permanent record.	
	3. Transport sample to laboratory.	3a. Transported to laboratory without delay. 3b. Sample iced if delay of starting test is greater than one hour. 3c. No longer than six hour delay from collection time to laboratory delivery. A two hour additional time period is allowable from taking the sample from the ice chest to completing membrane filtration first-day procedures.	
4. Preparation of Laboratory Data Sheet	1. Fill in data sheet to show sample information.	1a. Information needed should be on sample tag. 1b. Minimal information on data sheet should include:	

<table>
<tr><th>OPERATING PROCEDURES</th><th>STEP SEQUENCE</th><th>INFORMATION/OPERATING GOALS/SPECIFICATIONS</th><th>TRAINING GUIDE NOTES</th></tr>
<tr><td></td><td></td><td>source, date, collection time, name of sampler, name of lab analyst, assigned sample number, time of start of test, and sample volumes.</td><td></td></tr>
<tr><td></td><td>2. Select sample volumes and record on data sheet.</td><td>2a. According to fecal coliform density range predicted for the sample.
2b. For fecal coliforms per 100 ml in the range:

Expected range per 100 ml

<table>
<tr><th>From</th><th>To</th><th>Sample Volume in Milliliters</th></tr>
<tr><td>2000</td><td>- 6000</td><td>1</td></tr>
<tr><td>400</td><td>- 1200</td><td>5*</td></tr>
<tr><td>200</td><td>- 600</td><td>10*</td></tr>
<tr><td>100</td><td>- 300</td><td>20*</td></tr>
<tr><td>40</td><td>- 120</td><td>50</td></tr>
<tr><td>20</td><td>- 60</td><td>100</td></tr>
</table>

Volumes showing asterisk (*) should be those used as these will cover the range of counts to demonstrate compliance or non-compliance with effluent permit requirements.</td><td></td></tr>
<tr><td>5. Preparation of laboratory bench area</td><td>1. Disinfect laboratory bench area.</td><td>1a. Sponge, disinfectant solution, paper toweling.</td><td></td></tr>
<tr><td>6. Petri dish preparation</td><td>1. Set out required number of sterile petri dishes on laboratory bench.</td><td>1a. Plastic dishes are purchased in a pre-sterilized condition.</td><td></td></tr>
<tr><td></td><td>2. Place a sterile absorbant pad in each petri dish.</td><td>2a. Handle aseptically (not introducing bacterial contamination) using a forceps which has been stored in methanol or ethanol and flamed by passing the forceps quickly through a flame. keep approximately 1/2 of forceps in alcohol by using jar or beaker.</td><td></td></tr>
</table>

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
	3. Using a sterile pipet, pipet approximately 2 ml of M-FC medium over each absorbant pad.	3a. Amount does not have to be precise as 2 ml is an excess. 3b. Medium prepared and handled in accordance with A.12. 3c. Keep plates covered.	
	4. Gently tip uncovered and allow any excess medium to flow out of plates.	4a. Undue tapping or shaking may spill too much medium and leave plates without sufficient medium. 4b. Cover plate. It is now ready for use.	
	5. Label each dish with the sample volumes to be filtered.	5a. Use wax pencil or stick on label with pen. 5b. Label bottom (or base) of each plate. Pad will not fall when plate is inverted.	
7. Filtration procedure	1. Assemble filter assembly upon filtration flask.	1a. Sterile funnel units removed from wrapping. 1b. Using care to prevent contamination, such as would be caused by fingers, touching of units to equipment, etc. 1c. Unit should be connected to vacuum source and have a means of vacuum disconnection, such as by pinch clamp on the hose.	
	2. Place membrane filter on base of funnel apparatus.	2a. Funnel top removed carefully to avoid contamination. 2b. MF should be grid or inked side up. MF handled with flamed forceps and only by its outer 1/8 inch edge. 2c. Replace funnel top. Avoid over tightening	
	3. Deliver measured volume of a well shaken sample into the funnel.	3a. Well shaken to insure even distribution of bacteria. 3b. Poured gently into funnel, either by pipeting or by use of a presterilized graduated cylinder (use Kraft paper or foil hood). Avoiding splashing of sample, and if graduate cylinder is used, rinsed several times with small amounts of sterile buffered distilled water which are also poured into the funnel.	V.B.7.3

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
		3c. If small sample portions are to be used (less than 5 ml), a small amount of sterile buffered distilled must be added to the funnel prior to sample addition and then a gentle swirling of the funnel to distribute the bacteria present.	
	4. Apply suction.	4a. Vacuum applied only after sample has been completely delivered to funnel. 4b. Wait for complete evacuation of sample from funnel.	
	5. Rinse funnel.	5a. Three separate rinses with sterile buffered distilled water. Complete evacuation of water must occur between each application of rinse-water using about 20 ml for each rinse. 5b. Vacuum supply shut off after last rinse.	
	6. Remove MF.	6a. Handle gently with flamed forceps only on outer 1/8 inch of MF edge. 6b. Lifted gently from funnel base to break residual vacuum before lifting.	
	7. Reassemble funnel.	7a. Unit is ready for next sample and sterilization will not be required. 7b. If unit is not used within an hour it is advisable to re-sterilize. 7c. Handling of funnel top is critical in that no contamination should occur. Avoid handling in funnel surfaces that receive sample and do not lay on table that may have residual germicide. A ringstand with split ring or resting on the funnel top only on its base after hand lifting are recommended methods.	V.B.7.7
8. Plating procedure	1. Remove MF.	1a. This was done as part of the filtration procedure (B.7.6) and held with one hand as the funnel is reassembled (8.7.7).	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
	2. Remove cover from petri dish with prepared M-FC medium.	2a. With practice this can be done with one hand while the MF is held in the other. As an aid for easier operations, the plates can be organized in the order of plating and their tops can be loosened.	
	3. Plate MF over medium.	3a. Allow the side of the MF opposite the forceps to touch the saturated absorption pad, and with a rolling action, plate the entire membrane onto the pad. The grid (inked squares) must be up. 3b. Rolling action will tend to expel air bubbles from under the MF and allow an intimate contact between medium and MF. Air pockets must be removed for recovery of all fecal coliform organisms. 3b. Air pockets removed from underside of MF by gently lifting some or all of MF and re-rolling upon medium. At no time must a forceps be used to "smoothout" the surface of the MF. Any movement on the delicate surface of the MF by the forceps will result in a line growth of bacteria instead of the easily countable discrete colony form.	
9. Incubation procedure	1. Place plate/plates in protective plastic bag and incubate at 44.5°C.	1a. Completed plates must be incubated within 30 minutes from the time they are filtered. 1b. A variety of bags and bag closure devices exist. One of the simplest is a "Whirl-Pac" which has metal wire fold covers to keep rolled end sealed and leakproof. 1c. Bags containing plates must be totally immersed within the incubator's water. It is usually necessary to arrange some weight, such as a test tube rack, to keep the plates under water. 1d. All petri dishes must be incubated in the inverted position (pad, medium, and MF) now on top of the plate) so that droplets cannot fall on surface of MF.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
		1e. Observe immersed bag for a short time to observe that a constant bubbling action is not occuring to indicate bag leakage. Reseal if this is occurring and recheck. 1f. Allow to incubate for 24 hours ± 2.0 hours.	
C. Second Day Procedure 1. Counting procedure	1. Remove bag from water bath incubator.	1a. Be sure the incubation has been within the limits of 24 hours ± 2 hours. 1b. Handle carefully to avoid droplet splattering within plates.	
	2. Remove plates from plastic bag.	2a. Set plates on table so that colonies (growth) are visible.	
	3. Select plates which have from 20 to 60 colonies.	3a. This ability comes with experience, but plates which are overcrowded or the ones which have fewer colonies should be readily apparent. It is necessary only to record plate counts within these ranges. If this is not possible other counts can be used as described further.	
	4. Count fecal coliform colonies with microscopic aid.	4a. Binocular wide field dissecting microscope preferred. 4b. Use a 10-15 X magnification with flourescent lighting. 4c. Scan membrane with a back-and-forth movement over the grids, line by line, so as to cover the membrane completely without missing any area. 4d. All blue or blue-tinted (blue-green, purple, etc.) colonies are counted as fecal coliforms. Coloration may be deep or light and can be all over or partially cover the colony. Some can even have the coloring appear in flecks on the surface.	V.C.1.4

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
	5. Select colony count/counts to use. Use formula to calculate count per 100 ml.	5a. Formula	

Fecal Coliforms per 100 ml =

$$100 \times \frac{\text{colony count}}{\text{number of milliliters (mls) filtered}}$$

5b. Select colony count which falls within the 20-60 range:

Example

mls Filtered	No. Colonies
20	TNTC (indicates too numerous to count)
10	TNTC
5	35

Use: 35 colonies with a 5 ml sample volume

$$\text{Fecal coliform per 100 ml} = 100 \times \frac{35}{5} = 700$$

5c. If more than one plate has colony numbers within the range, add the results.

Example

mls Filtered	No. Colonies
20	45
10	23
5	9

20 ml + 10 ml = 30 ml

45 colonies + 23 colonies = 68

$$\text{Fecal coliforms per 100 ml} = 100 \times \frac{68}{30} = 227$$

Use: 230 (nearest two significant figures)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES

5d. If no counts were obtained within these ranges, the following procedure should be followed:

All above 60 Colonies
Use that count which is closer to the maximum 60 count.

Example

20 ml	TNTC
10 ml	150
5 ml	72

Use: 72 colonies with a 5 ml sample volume
fecal coliforms/100 ml = $100 \times \frac{72}{5} = 1440$

or 1400 fecal coliform per 100 ml

All below 20 Colonies
Use that count which is closer to the 20 count.

Example

20 ml	15
10 ml	8
5 ml	0

Use: 15 colonies with a 20 ml sample volume which gives 75/100 ml fecal coliforms.

All Plates with a Zero Count of Fecal Coliform

Assume that the largest volume delivered has one colony. Use this in calculations and call the result <(less than). If all three plates show a zero count the fecal coliform count would be < 5 (calculation: $100 \times \frac{1}{20}$).

299

300

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
	6. Record colony counts on data sheet.	6. There is no such thing as a "standard" data sheet for bacteriological tests. A simplified data sheet is shown below:	

Fecal Coliform Test
Membrane Filter (MF) Procedure

Sample Type ____________ Lab. No. ________

Station ________ Description ____________

Collection Date ______ Time ______ APM. Temp. ________

Received ________ APM. Examined ________ APM.

pH ________ Observations ____________

mls Filtered	Colony Count	Remarks

Results:
Fecal Coliform (MF)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
	7. Record fecal coliform count/100 ml.	7a. Record on designated data sheet for your agency. 7b. Record to nearest two significant figures. Examples 266.6 will be 270 20.09 will be 20 299.4 will be 300	

SECTION	TOPIC
I	Introduction
II*	Educational Concepts - Mathematics
III	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field and Laboratory Equipment
VI*	Field and Laboratory Reagents
VII	Field and Laboratory Analysis
VIII	Safety
IX	Records and Reports

Training guide materials are presented here under the headings marked. These standardized headings are used throughout this series of procedures.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES

A.12.5 Since 3.7 grams of MFC powdered medium and 1 ml of 1% Rosolic Acid is required to prepare 100 ml of MFC broth, it is possible to calculate weights and volumes to prepare any requirement based upon the number of plates desired. Calculations are based on knowing the above figures and the requirement of 2.0 ml of broth for each plate.

For rapid calculations the following two formulas can be used:

(1) No. of plates desired X 0.074 = Grams MFC
(2) No. of plates desired X 0.02 = ML Rosolic Acid

EXAMPLE

If 125 plates of MFC are required:

125 X 0.074 = 9.25

= 9.3 grams MFC medium required

125 X 0.02 = 2.5 mls 1% Rosolic Acid required

Note: Due to the difficulties involved in weighing very small portions as, for instance, .074 grams of MFC for one plate requirement, it would be wise to prepare at least 10 plates (.7 gr. MFC and 0.2 ml Rosolic Acid) as a minimum requirement.

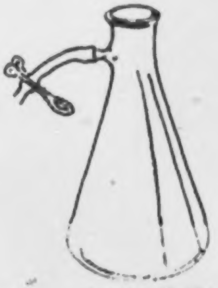
	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.1.1	Incubator should be kept out of drafts or direct sunlight in order to prevent temperature inside the incubator from changing outside the temperature range specified (44.5°C ± 0.2°C).	Standard Methods for the Examination of Water and Wastewater 14th Ed. (1975) APHA, WPCF, AWWA, p.880 ff. (Hereafter refferred to as: Std. Meth. 14: (Page No.)
A.1.2	An accurate solid heat sink may be used. This is constructed of a solid aluminum block and does not contain water for transference of heat. Plastic bags, for this reason, are no longer required when using this type of incubator. Since there are no provisions for a high humidity chamber in this type of incubator, it is important to use only the types of petri dishes having a tight attachment of cover-to-base thus preventing loss of moisture during the incubation.	Std. Meth. 14: 937
A.1.3	Mercury bulb thermometer usually used in most incubators and a recording thermometer is acceptable. Thermometers must be calibrated against a mercury bulb thermometer which is (or calibrated against) a National Bureau of Standards issued and used with the certificate and correction chart.	
A.3	Autoclaves differ greatly in design and in method of operation. Some are almost like home-style pressure coolers; others are almost fully automatic.	Std. Meth. 14: 881
A.4	Distilled water must not contain substances preventing bacterial growth or be highly nutritive. There are required procedures to testing distilled water and should be undertaken only by professional bacteriologists or in laboratories where this is done regularly.	Std.Meth. 14: 888
A.6.2	Glassware can be checked for bacteriostatic or inhibitory residues by a bacteriological test procedure which, like the distilled water suitability test, should be undertaken only by professional bacteriologists or in laboratories where this test is done on a regular basis.	Std. Meth. 14: 885

Field and Laboratory Equipment

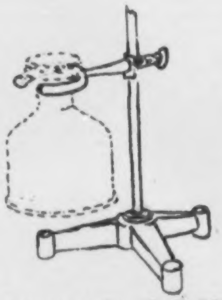
Section V

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.9.1-6	Wide-mouth, glass-stoppered bottles suggested, but other styles accepted. Bottle must be heat stable to sterilizing conditions and not be toxic or nutritive to organisms natural to the sample. If glass-stoppered bottles are used, a strip of paper should be placed in the neck of the bottle before placing the stopper in place in preparation for sterilization. This prevents the glass stopper from "freezing" in place during sterilization. The paper strip is descarded at the time of sample collection.	Std. Meth. 14: 884 14: 904
A.10.1-5	This procedure is described in terms of reusable glass pipets. However, single-service, pre-packaged, glass or plastic pipets may be used. In the case of single-service pipets, they will be sterile when purchased, are used one time, and discarded after use. Accordingly, in the step-by-step procedures, disregard any instructions about pipet preparation if these pipets are used.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
B.7.3	If graduate is labeled TC (To Contain), follow the rinsing procedure. If it is labeled TD (To Deliver) slowly add the complete sample, gently tap off the last drop and do not rinse.	
B.7.7	When the filter holding unit is disassembled after sample filtration, the worker's hands must be free to manipulate the membrane filter. Upon disassembly of the filter holding unit, many workers place the funnel element, inverted, on the laboratory bench. Some workers, to prevent bacterial contamination, prefer a rack or a support to keep the funnel element from any possible source of contamination. A split ring on a ring stand is a convenient rack for this purpose.	

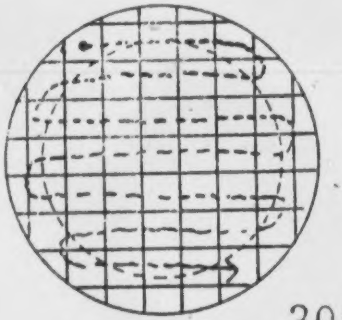


SUCTION FLASK



RING STAND WITH SPLIT RING

C.1.4	The dashed circle indicates the effective filtering area. The dashed back-and-forth line indicates the colony counting pathway.	



	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.12.2	Rosolic acid may be omitted from the medium if minimal background colony counts occur and equivalent results are obtained without it.	Std. Meth. 14: 894

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

CALCULATION OF THE GEOMETRIC MEAN OF COLIFORM COUNTS

by the

USE OF LOGARITHMS

as applied in

WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

Developed by the

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

EFFLUENT MONITORING PROCEDURE: Calculation of the Geometric Mean of Coliform Counts by the Use of Logarithms

This procedure was developed by:

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POSITION Mathematical Statistician

EDUCATION & TECHNICAL BACKGROUND

BS - St. Louis University

MS - St. Louis University

4 years - U.S. Army

3 years - Operations Research Analyst

7 years - University Professor - Mathematics

12 years - Statistical consultant and training of Federal, State, and Local personnel in the principles and practice of statistical analysis

EFFLUENT MONITORING PROCEDURE : Calculation of the Geometric Mean of Coliform Counts by the Use of Logarithms

1. The object of this procedure is the calculation of the geometric mean of fecal coliform counts when using logarithms.

2. Brief description of the procedure.

   How to use logarithms (or logs) and find the geometric mean (or GM) of n fecal coliform counts, where each count is greater than or equal to one.

   Let the first fecal coliform count = N_1

   Let the second fecal coliform count = N_2

   etc.

   Let the last fecal coliform count = N_n

   Let n equal the total number of such fecal coliform counts or n = sample size. The formula for the GM when using logs is:

$$\text{GM (of } N_1, N_2, \ldots, N_n) = \text{anti-log}\left[\frac{\log N_1 + \log N_2 + \ldots + \log N_n}{n}\right]$$

   In order to complete the calculations on the right hand side of the equation, four operations are necessary.

   A. Determine the log for each of the n fecal coliform counts.
   B. Add or sum the n logs
   C. Divide the sum by sample size equal to n
   D. Find the anti-log of the answer to step C.

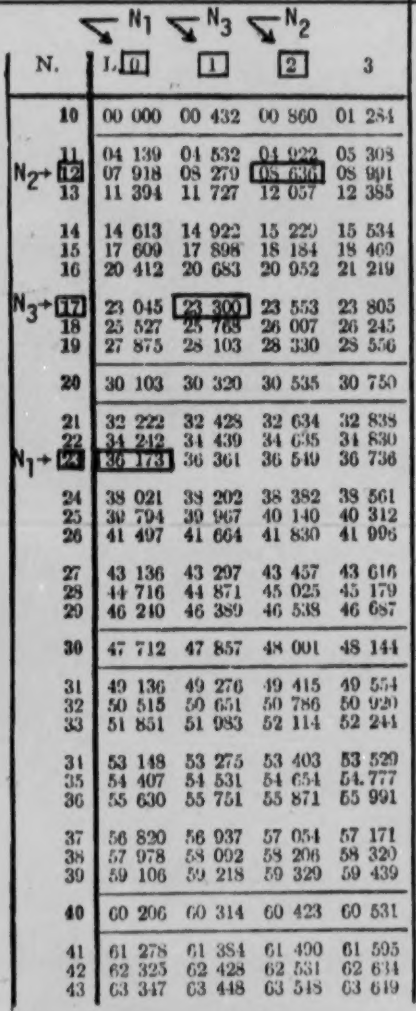
EFFLUENT MONITORING PROCEDURE: Calculation of the Geometric Mean of Coliform Counts by the Use of Logarithms

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Finding the log of a fecal coliform count	1. Determine d = number of digits to the left of the decimal point	Examples 1a. if N_1 = 23 then d = 2 1b. if N_2 = 122 then d = 3 1c. if N_3 = 17,100 then d = 5	
	2. Calculate C = d-1 (C is called the characteristic by mathematicians)	2a. if N_1 = 23 then d = 2 and C = 2-1 = 1 2b. if N_1 = 122 then d = 3 and C = 2 2c. if N_1 = 17,100 then d = 5 and C = 4	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. (Cont'd.)	3. Locate N in the table margin. The first two digits in the first column in the left margin and the third digit in the first row. Note that trailing zero's can be added or deleted in order to have the necessary three digits for entry into the tables.	3a. For N_1 = 23, there are only 2 digits so we must add a trailing zero. Locate 23 or the first two digits in the left margin and the third or last digit 0, in the top row. 3b. For N_2 = 122, the first two digits or 12 are located in the left margin and the third digit or 2 is located in the top row. 3c. For N_3 = 17,100 the first two digits or 17 are located in the left margin. The third digit is a 1 and is located in the first row. All digits after the third are deleted or these trailing zeros are ignored.	

	N_1	N_3	N_2	
N.	L. 0	1	2	3
10	00 000	00 432	00 860	01 284
11	04 139	04 532	04 922	05 308
N_2→ 12	07 918	08 279	08 636	08 991
13	11 394	11 727	12 057	12 385
14	14 613	14 922	15 229	15 534
15	17 609	17 898	18 184	18 469
16	20 412	20 683	20 952	21 219
N_3→ 17	23 045	23 300	23 553	23 805
18	25 527	25 768	26 007	26 245
19	27 875	28 103	28 330	28 556
20	30 103	30 320	30 535	30 750
21	32 222	32 428	32 634	32 838
22	34 242	34 439	34 635	34 830
N_1→ 23	36 173	36 361	36 549	36 736
24	38 021	38 202	38 382	38 561
25	39 794	39 967	40 140	40 312
26	41 497	41 664	41 830	41 996
27	43 136	43 297	43 457	43 616
28	44 716	44 871	45 025	45 179
29	46 240	46 389	46 538	46 687
30	47 712	47 857	48 001	48 144
31	49 136	49 276	49 415	49 554
32	50 515	50 651	50 786	50 920
33	51 851	51 983	52 114	52 244
34	53 148	53 275	53 403	53 529
35	54 407	54 531	54 654	54.777
36	55 630	55 751	55 871	55 991
37	56 820	56 937	57 054	57 171
38	57 978	58 092	58 206	58 320
39	59 106	59 218	59 329	59 439
40	60 206	60 314	60 423	60 531
41	61 278	61 384	61 490	61 595
42	62 325	62 428	62 531	62 634
43	63 347	63 448	63 548	63 649

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. (Cont'd.)	4. Read the 5 digit number within the body of the table at the intersection of the row and column determined in step 3. We label this number M. The mathematicians call it the mantissa.	4a. For N_1 = 23 then M = 36173 4b. For N_2 = 122 then M = 08636 4c. For N_3 = 17,100 then M = 23300	

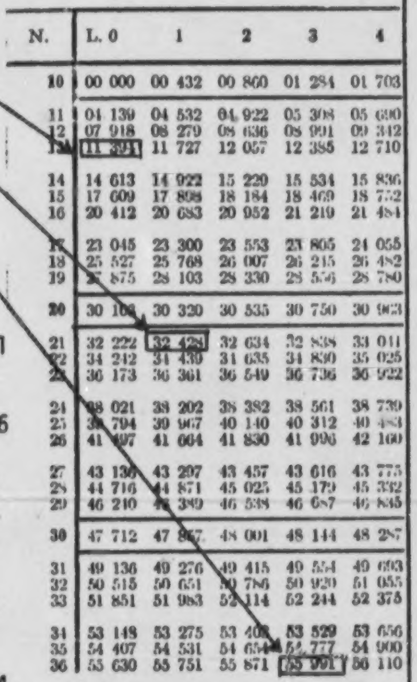

N_1
N_3
N_2
N_2→
N_3→
N_1→

N.	L. 0	1	2	3
10	00 000	00 432	00 860	01 284
11	04 139	04 532	04 922	05 308
12	07 918	08 279	08 636	08 991
13	11 394	11 727	12 057	12 385
14	14 613	14 922	15 229	15 534
15	17 609	17 898	18 184	18 469
16	20 412	20 683	20 952	21 219
17	23 045	23 300	23 553	23 805
18	25 527	25 768	26 007	26 245
19	27 875	28 103	28 330	28 556
20	30 103	30 320	30 535	30 750
21	32 222	32 428	32 634	32 838
22	34 242	34 439	34 635	34 830
23	36 173	36 361	36 549	36 736
24	38 021	38 202	38 382	38 561
25	39 794	39 967	40 140	40 312
26	41 497	41 664	41 830	41 996
27	43 136	43 297	43 457	43 616
28	44 716	44 871	45 025	45 179
20	46 240	46 389	46 538	46 687
30	47 712	47 857	48 001	48 144
31	49 136	49 276	49 415	49 554
32	50 515	50 651	50 786	50 920
33	51 851	51 983	52 114	52 244
34	53 148	53 275	53 403	53 529
35	54 407	54 531	54 654	54 777
36	55 630	55 751	55 871	55 991
37	56 820	56 937	57 054	57 171
38	57 978	58 092	58 206	58 320
39	59 106	59 218	59 329	59 439
40	60 206	60 314	60 423	60 531
41	61 278	61 384	61 490	61 595
42	62 325	62 428	62 531	62 634
43	63 347	63 448	63 548	63 649

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. (Cont'd.)	5. Log N = C.M	5a. Log 23 = 1.36173 5b. Log 122 = 2.08636 5c. Log 17,100 = 4.23300 ↑ C obtained in step 2 ↖ M obtained in step 4	
B. Sum the n logs	1. It is assumed that addition is known.		
C. Divide the sum of the logs by n, sample size	1. It is assumed that division is known		
D. Finding the anti-log of a positive number	1. Determine M = the number to the right of the decimal point.	1a. If we want the anti-log of 3.11394 then M = 11394 1b. If we want the anti-log of 2.32428 then M = 32428 1c. If we want the anti-log of 2.56036 then M = 56036	

EFFLUENT MONITORING PROCEDURE: Calculation of the Geometric Mean of Coliform Counts by the Use of Logarithms

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. (Cont'd.)	2. Locate M or its nearest value within the body of the table.	2a. M = 11394 2b. M = 32428 2c. M = 56036 (Nearest value is 55991)	
	3. Read N = rc at the intersection of row (r) and column (c) where M was located in step 2. Note rc is the 2 digit r and the one digit c written side by side and are not an indicated multiplication.	3a. If M = 11394 then N = rc = 130 since r = 13 and c = 0 3b. If M = 32428 then N = 211 since r = 21 and c = 1 3c. If M = 56036 then N = 363 since r = 36 and c = 3	
	4. Determine C = number to the left of the decimal point.	4a. For the anti-log of 3.11394 then C = 3 4b. For the anti-log of 2.32428 then C = 2 4c. For the anti-log of 2.56036 then C = 2	
	5. Calculate d = C+1	5a. If C = 3, then d = 4 5b. If C = 2, then d = 3 5c. If C = 2, then d = 3	
	6. Locate the decimal point for the number determined in step 3 so that there are d digits to the left of the decimal point. Note trailing zeros can be added or deleted.	6a. Anti-log 3.11394 = 1300. The 130 was determined in step 3a and the decimal point was placed so that 4 digits (see 5a) are to the left of it. In this case a trailing zero was added.	



N.	L. 0	1	2	3	4
10	00 000	00 432	00 860	01 284	01 703
11	04 139	04 532	04 922	05 308	05 690
12	07 918	08 279	08 636	08 991	09 342
13	11 394	11 727	12 057	12 385	12 710
14	14 613	14 922	15 229	15 534	15 836
15	17 609	17 898	18 184	18 469	18 752
16	20 412	20 683	20 952	21 219	21 484
17	23 045	23 300	23 553	23 805	24 055
18	25 527	25 768	26 007	26 245	26 482
19	27 875	28 103	28 330	28 556	28 780
20	30 103	30 320	30 535	30 750	30 963
21	32 222	32 428	32 634	32 838	33 041
22	34 242	34 439	34 635	34 830	35 025
23	36 173	36 361	36 549	36 736	36 922
24	38 021	38 202	38 382	38 561	38 739
25	39 794	39 967	40 140	40 312	40 483
26	41 497	41 664	41 830	41 996	42 160
27	43 136	43 297	43 457	43 616	43 775
28	44 716	44 871	45 025	45 179	45 332
29	46 240	46 389	46 538	46 687	46 835
30	47 712	47 857	48 001	48 144	48 287
31	49 136	49 276	49 415	49 554	49 693
32	50 515	50 651	50 786	50 920	51 055
33	51 851	51 983	52 114	52 244	52 375
34	53 148	53 275	53 403	53 529	53 656
35	54 407	54 531	54 654	54 777	54 900
36	55 630	55 751	55 871	55 991	56 110

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. (Cont'd.)	6. (Cont'd.)	6b. Anti-log 2.32428 = 211. Step 3b followed by step 5b. 6c. Anti-log 2.56036 = 363. By combining steps 3c through 5c.	

—Common logarithms of numbers—

N.	L. 0	1	2	3	4	5	6	7	8	9
10	00 000	00 432	00 860	01 284	01 703	02 119	02 531	02 938	03 342	03 743
11	04 139	04 532	04 922	05 308	05 690	06 070	06 446	06 819	07 188	07 555
12	07 918	08 279	08 636	08 991	09 342	09 691	10 037	10 380	10 721	11 059
13	11 394	11 727	12 057	12 385	12 710	13 033	13 354	13 672	13 988	14 301
14	14 613	14 922	15 229	15 534	15 836	16 137	16 435	16 732	17 026	17 319
15	17 609	17 898	18 184	18 469	18 752	19 033	19 312	19 590	19 866	20 140
16	20 412	20 683	20 952	21 219	21 484	21 748	22 011	22 272	22 531	22 789
17	23 045	23 300	23 553	23 805	24 055	24 304	24 551	24 797	25 042	25 285
18	25 527	25 768	26 007	26 245	26 482	26 717	26 951	27 184	27 416	27 646
19	27 875	28 103	28 330	28 556	28 780	29 003	29 226	29 447	29 667	29 885
20	30 103	30 320	30 535	30 750	30 963	31 175	31 387	31 597	31 806	32 015
21	32 222	32 428	32 634	32 838	33 041	33 244	33 445	33 646	33 846	34 044
22	34 242	34 439	34 635	34 830	35 025	35 218	35 411	35 603	35 793	35 984
23	36 173	36 361	36 549	36 736	36 922	37 107	37 291	37 475	37 658	37 840
24	38 021	38 202	38 382	38 561	38 739	38 917	39 094	39 270	39 445	39 620
25	39 794	39 967	40 140	40 312	40 483	40 654	40 824	40 993	41 162	41 330
26	41 497	41 664	41 830	41 996	42 160	42 325	42 488	42 651	42 813	42 975
27	43 136	43 297	43 457	43 616	43 775	43 933	44 091	44 248	44 404	44 560
28	44 716	44 871	45 025	45 179	45 332	45 484	45 637	45 788	45 939	46 090
29	46 240	46 389	46 538	46 687	46 835	46 982	47 129	47 276	47 422	47 567
30	47 712	47 857	48 001	48 144	48 287	48 430	48 572	48 714	48 855	48 996
31	49 136	49 276	49 415	49 554	49 693	49 831	49 969	50 106	50 243	50 379
32	50 515	50 651	50 786	50 920	51 055	51 188	51 322	51 455	51 587	51 720
33	51 851	51 983	52 114	52 244	52 375	52 504	52 634	52 763	52 892	53 020
34	53 148	53 275	53 403	53 529	53 656	53 782	53 908	54 033	54 158	54 283
35	54 407	54 531	54 654	54 777	54 900	55 023	55 145	55 267	55 388	55 509
36	55 630	55 751	55 871	55 991	56 110	56 229	56 348	56 467	56 585	56 703
37	56 820	56 937	57 054	57 171	57 287	57 403	57 519	57 634	57 749	57 864
38	57 978	58 092	58 206	58 320	58 433	58 546	58 659	58 771	58 883	58 995
39	59 106	59 218	59 329	59 439	59 550	59 660	59 770	59 879	59 988	60 097
40	60 206	60 314	60 423	60 531	60 638	60 746	60 853	60 959	61 066	61 172
41	61 278	61 384	61 490	61 595	61 700	61 805	61 909	62 014	62 118	62 221
42	62 325	62 428	62 531	62 634	62 737	62 839	62 941	63 043	63 144	63 246
43	63 347	63 448	63 548	63 649	63 749	63 849	63 949	64 048	64 147	64 246
44	64 345	64 444	64 542	64 640	64 738	64 836	64 933	65 031	65 128	65 225
45	65 321	65 418	65 514	65 610	65 706	65 801	65 896	65 992	66 087	66 181
46	66 276	66 370	66 464	66 558	66 652	66 745	66 839	66 932	67 025	67 117
47	67 210	67 302	67 394	67 486	67 578	67 669	67 761	67 852	67 943	68 034
48	68 124	68 215	68 305	68 395	68 485	68 574	68 664	68 753	68 842	68 931
49	69 020	69 108	69 197	69 285	69 373	69 461	69 548	69 636	69 723	69 810
50	69 897	69 984	70 070	79 157	70 243	70 329	70 415	70 501	70 586	70 672
N.	L. 0	1	2	3	4	5	6	7	8	9

TM 5-236
War Department July 10, 1940

—*Common logarithms of numbers*—

N.	L. 0	1	2	3	4	5	6	7	8	9
50	69 897	69 984	70 070	70 157	70 243	70 329	70 415	70 501	70 586	70 672
51	70 757	70 842	70 927	71 012	71 096	71 181	71 265	71 349	71 433	71 517
52	71 600	71 684	71 767	71 850	71 933	72 016	72 099	72 181	72 263	72 346
53	72 428	72 509	72 591	72 673	72 754	72 835	72 916	72 997	73 078	73 159
54	73 239	73 320	73 400	73 480	73 560	73 640	73 719	73 799	73 878	73 957
55	74 036	74 115	74 194	74 273	74 351	74 429	74 507	74 586	74 663	74 741
56	74 819	74 896	74 974	75 051	75 128	75 205	75 282	75 358	75 435	75 511
57	75 587	75 664	75 740	75 815	75 891	75 967	76 042	76 118	76 193	76 268
58	76 343	76 418	76 492	76 567	76 641	76 716	76 790	76 864	76 938	77 012
59	77 085	77 159	77 232	77 305	77 379	77 452	77 525	77 597	77 670	77 743
60	77 815	77 887	77 960	78 032	78 104	78 176	78 247	78 319	78 390	78 462
61	78 533	78 604	78 675	78 746	78 817	78 888	78 958	79 029	79 099	79 169
62	79 239	79 309	79 379	79 449	79 518	79 588	79 657	79 727	79 796	79 865
63	79 934	80 003	80 072	80 140	80 209	80 277	80 346	80 414	80 482	80 550
64	80 618	80 686	80 754	80 821	80 889	80 956	81 023	81 090	81 158	81 224
65	81 291	81 358	81 425	81 491	81 558	81 624	81 690	81 757	81 823	81 889
66	81 954	82 020	82 086	82 151	82 217	82 282	82 347	82 413	82 478	82 543
67	82 607	82 672	82 737	82 802	82 866	82 930	82 995	83 059	83 123	83 187
68	83 251	83 315	83 378	83 442	83 506	83 569	83 632	83 696	83 759	83 822
69	83 885	83 948	84 011	84 073	84 136	84 198	84 261	84 323	84 386	84 448
70	84 510	84 572	84 634	84 696	84 757	84 819	84 880	84 942	85 003	85 065
71	85 126	85 187	85 248	85 309	85 370	85 431	85 491	85 552	85 612	85 673
72	85 733	85 794	85 854	85 914	85 974	86 034	86 094	86 153	86 213	86 273
73	86 332	86 392	86 451	86 510	86 570	86 629	86 688	86 747	86 806	86 864
74	86 923	86 982	87 040	87 099	87 157	87 216	87 274	87 332	87 390	87 448
75	87 506	87 564	87 622	87 679	87 737	87 795	87 852	87 910	87 967	88 024
76	88 081	88 138	88 195	88 252	88 309	88 366	88 423	88 480	88 536	88 593
77	88 649	88 705	88 762	88 818	88 874	88 930	88 986	89 042	89 098	89 154
78	89 209	89 265	89 321	89 376	89 432	89 487	89 542	89 597	89 653	89 708
79	89 763	89 818	89 873	89 927	89 982	90 037	90 091	90 146	90 200	90 255
80	90 309	90 363	90 417	90 472	90 526	90 580	90 634	90 687	90 741	90 795
81	90 849	90 902	90 956	91 009	91 062	91 116	91 169	91 222	91 275	91 328
82	91 381	91 434	91 487	91 540	91 593	91 645	91 698	91 751	91 803	91 855
83	91 908	91 960	92 012	92 065	92 117	92 169	92 221	92 273	92 324	92 376
84	92 428	92 480	92 531	92 583	92 634	92 686	92 737	92 788	92 840	92 891
85	92 942	92 993	93 044	93 095	93 146	93 197	93 247	93 298	93 349	93 399
86	93 450	93 500	93 551	93 601	93 651	93 702	93 752	93 802	93 852	93 902
87	93 952	94 002	94 052	94 101	94 151	94 201	94 250	94 300	94 349	94 399
88	94 448	94 498	94 547	94 596	94 645	94 694	94 743	94 792	94 841	94 890
89	94 939	94 988	95 036	95 085	95 134	95 182	95 231	95 279	95 328	95 376
90	95 424	95 472	95 521	95 569	95 617	95 665	95 713	95 761	95 809	95 856
91	95 904	95 952	95 999	96 047	96 095	96 142	96 190	96 237	96 284	96 332
92	96 379	96 426	96 473	96 520	96 567	96 614	96 661	96 708	96 755	96 802
93	96 848	96 895	96 942	96 988	97 035	97 081	97 128	97 174	97 220	97 267
94	97 313	97 359	97 405	97 451	97 497	97 543	97 589	97 635	97 681	97 727
95	97 772	97 818	97 864	97 909	97 955	98 000	98 046	98 091	98 137	98 182
96	98 227	98 272	98 318	98 363	98 408	98 453	98 498	98 543	98 588	98 632
97	98 677	98 722	98 767	98 811	98 856	98 900	98 945	98 989	99 034	99 078
98	99 123	99 167	99 211	99 255	99 300	99 344	99 388	99 432	99 476	99 520
99	99 564	99 607	99 651	99 695	99 739	99 782	99 826	99 870	99 913	99 957
100	00 000	00 043	00 087	00 130	00 173	00 217	00 260	00 303	00 346	00 389
N.	L. 0	1	2	3	4	5	6	7	8	9

EFFLUENT MONITORING PROCEDURE: Calculation of the Geometric Mean of Coliform Counts by the Use of Logarithms

An example of the calculations for operating procedure A, B, C, and D follows.

$$GM\ (23,\ 122,\ 17100) = \text{Anti-log}\left[\frac{\log 23 + \log 122 + \log 17100}{3}\right]$$

(see A5a, 5b, 5c)

$$GM\ (23,\ 122,\ 17100) = \text{Anti-log}\left[\frac{1.36173 + 2.08636 + 4.23300}{3}\right]$$

(See procedure B and C)

$$GM\ (23,\ 122,\ 17100) = \text{Anti-log } 2.56036$$

(See D6c)

$$GM\ (23,\ 122,\ 17100) = 363.$$

The following practice problems should be solved to make sure that the program of action is mastered.

1) GM (1, 4) = 2

2) GM (1, 10, 100) = 10

3) GM (10, 10, 10) = 10

Some checks for gross errors.

1) GM lies between the largest and smallest value. For the problem GM (23, 122, 17100) = 363 the largest = 17,100 and the smallest = 23. Since 363 lies between these two, there is no gross error.

2) GM is less than the arithmatic mean* (AM). $AM = \frac{23 + 122 + 17100}{3} = 5748.3$

GM = 363 is less than AM = 5748.3. Hence, there is no gross error.

---

*GM=AM if all coliform counts are equal as illustrated in practice problem number 3.

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

MEASUREMENT OF FLOW IN AN OPEN CHANNEL BY PARSHALL FLUME

as applied in

WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

Developed by the

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

EFFLUENT MONITORING PROCEDURE: Flow Measurement in an Open Channel by Parshall Flume

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Industrial Wastes Consultant, Technical Advisory and
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Participation in and Direction of numberous in-plant industrial waste surveys and stream studies in New York, Colorado, New Mexico, Maine, Utah

With National Training & Operational Technology Center, September 1969 to date.

EFFLUENT MONITORING PROCEDURE: Flow Measurement in an Open Channel by Parshall Flume

1. Objective: To enable the student to obtain the flow rate in an open channel by means of a pre-installed Parshall Flume.

2. Description of Procedure:

   The depth of liquid is measured at a stipulated point (or points) within the Flume. This measurement is then used to obtain the rate of flow in the channel.

   a. This Procedure deals specifically with 6-inch through 8-foot flumes, since practically all wastewater treatment plant influent and effluent flows can be measured by flumes of this size. Operating principles of larger and smaller size flumes are exactly the same. For these latter however, some differences in procedures are involved, consisting of a change of location for measurement of the downstream head, and use of different discharge tables.

   b. Flows obtained by visual observation of liquid depth are considered herein. Use of devices which automatically provide a continuous record of either head or flow is not included.

General Description of Equipment used in the Procedure:

1) Parshall Flume.

2) Means for visually observing depth of flow, such as a staff gage or a float gage.

EFFLUENT MONITORING PROCEDURE: Flow Measurement in an Open Channel by Parshall Flume

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Basic Elements			
1. Units of Flow Measurement			II.A.1 (p. 11)
2. Description of Process			I.A.2 (p. 10)
3. Structure of Flume			V.A.3 (p. 24)
4. Terminology and Definitions			V.A.4 (p. 25)
5. Operating Principles			V.A.5 (p. 26)
6. Staff Gage			V.A.6 (p. 27)
7. Float Gage			V.A.7 (p. 27)
B. Preparation for Measurement			
1. Physical Conditions	1. Observe flow upstream of flume	1a. Reasonably smooth or streamline flow. 1b. Flow distributed reasonably uniformly across channel	III.B.1.1 (p. 22)
	2. Remove any objects causing disturbance of flow		
	3. Inspect flume for deposits of solids	3a. No build-up of sediment in structure	III.B.1.3 (p. 22)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
	4. Determine flow condition	4a. Free flow or submerged flow.	III.B.1.4 (p. 22)
	5. Inspect stilling well, clean if necessary	5a. Connection to channel not clogged. 5b. No deposits 5c. No objects interfering with float	III.B.1.5 (p. 23)
C. Flow Measurement - Free-Flow Condition, Using Staff Gage			
1. Determination of upstream head, H_a	1. Read gage division at which liquid surface intersects gage.	1a. To nearest division.	II.C.1 (p. 12)
	2. Calculate H_a	2a. From staff gage reading.	
2. Determination of flow rate.	1. Use appropriate table.	1a. In unit desired.	II.C.2 (p. 15)
D. Flow Measurement - Free-Flow Condition, Using Float Gage			
1. Determination of upstream head, H_a	1. Read tape division opposite index on float gage	1a. To nearest division.	II.D.1 (p. 16)
	2. Calculate H_a	2a. From float gage reading	
2. Determination of flow rate	1. Use appropriate table	1a. In unit desired	II.C.2 (p. 15)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Flow Measurement - Submerged-Flow Condition, using Staff Gages			
1. Determination of upstream head, H_a	1. Read gage division at which liquid surface intersects gage.	1a. To nearest division. 1b. At the same time as for H_b	II.C.1 (p. 12)
	2. Calculate H_a	2a. From staff gage reading.	
2. Determination of downstream head, H_b	1. Read gage division at which liquid surface intersects gage.	1a. To nearest division. 1b. At the same time as for H_a	II.C.1 (p. 12)
	2. Calculate H_b	2a. From staff gage reading.	
3. Determination of flow rate.	1. Calculate percent submergence	1a. Percent submergence = $\frac{H_b}{H_a}$ x 100	
	2. Consult appropriate chart or table.		II.E.3.2 (p. 17)
	3. Read submerged flow value, or obtain correction to be applied to free-flow value.		
	4. When a correction factor is obtained, use H_a and find free-flow from Table 1.		
	5. Multiply this free-flow value by the correction factor to obtain the submerged flow.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Flow Measurement - Submerged-Flow Condition, using Float Gages			
1. Determination of upstream head, H_a	1. Read tape division opposite index on gage.	1a. To nearest division. 1b. At the same time as for H_b	II.D.1 (p. 16)
	2. Calculate H_a	2a. From float gage reading.	
2. Determination of downstream head, H_b	1. Read tape division opposite index on gage.	1a. To nearest division 1b. At the same time as for H_a	II.D.1 (p. 16)
	2. Calculate H_b	2a. From float gage reading	
3. Determination of flow rate.	1. Calculate percent submergence.	1a. Percent submergence = $\frac{H_b}{H_a} \times 100$	
	2. Consult appropriate chart or table.		II.E.3.2 (p. 17)
	3. Read submerged flow value directly or obtain correction to be applied to free-flow value.		
	4. When a correction factor is obtained, use H_a and find free-flow from Table 1.		
	5. Multiply this free-flow value by the correction factor to obtain the submerged flow.		

TRAINING GUIDE

SECTION	TOPIC
I*	Introduction
II*	Educational Concepts - Mathematics
III*	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field & Laboratory Equipment
VI	Field & Laboratory Reagents
VII	Field & Laboratory Analysis
VIII	Safety
IX	Records & Reports

Training guide materials are presented here under the headings marked. These standardized headings are used throughout this series of procedures

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.2	Flow of a liquid in an open channel can be measured in many cases by means of a specially-shaped section known as a Parshall Flume. The flume can be constructed as part of the channel, or installed later either temporarily or permanently. The depth of the flowing liquid is determined at a specific point, or points, in the flume. The measured depth, or depths, can then be used to obtain the rate of flow of the liquid in the channel.	1. Handbook of Hydraulics, King, H. W., McGraw-Hill, NY, 3rd Ed., 1939 2. Water Measurement Manual, US Dept. of the Interior, Bureau of Reclamation, Denver, CO, 2nd Ed., 1967 3. Stevens Water Resources Data Book, Leupold & Stevens, Inc., Box 688, Beaverton, OR 97005, 2nd. Ed., $4.00

A.1

Flows - Units of Expression

I. Flow, or Flow Rate, or Discharge.

All of these terms are commonly used to refer to the quantity of liquid passing a point in a certain time interval.

II. Quantity of liquid can be expressed in a number of ways. Common units are the gallon (Gal) and the cubic foot (cu.ft., $ft.^3$). To change from one of these measures to another, use the table below:

Multiply	by	To obtain
cu.ft.	7.5	Gal.
Gal.	0.134	cu.ft.

III. Flow is usually expressed in these units:

Gallons per minute (GPM)
Million gallons per day (MGD)
Cubic feet per second (cfs, Sec.-ft.)

To change from one of these units to another, use this table:

Multiply	by	To obtain
cfs	0.646	MGD
MGD	1.55	cfs
cfs	448.8	GPM
GPM	0.0022	cfs
MGD	694.4	GPM
GPM	0.00144	MGD

IV. Flow data is needed to calculate the quantity of constituents discharged in a plant effluent. Formulas are--

$$\text{lb/day} = \text{MGD} \times \text{mg/l} \times 8.34$$
$$\text{Kg/day} = \text{MGD} \times \text{mg/l} \times 3.78$$

TRAINING GUIDE NOTE | REFERENCES/RESOURCES

C.1 The head H_a is the vertical distance from the crest of the flume (floor of the converging section) to the liquid surface, at the stipulated point in the converging section. Head H_b is the corresponding distance, as measured at the stipulated point in the throat of the flume. Both of these measurements are referenced to the same point, i.e., the elevation of the crest of the flume. Consequently, all equipment and devices used to measure these heads must also be referenced to the crest elevation.

When a staff gage is used to obtain these heads, it may be attached to the inside face of the flume, or placed in a stilling well. In the former case, only an approximate head determination is usually possible, because of waves and rapid water level fluctuations at the upstream gage, and turbulent conditions at the downstream gage.

Determination of head using the staff gage is illustrated below for the various conditions which will be met.

Case I - Initial gage mark 0.00 ft.

The gage may be installed in either of three positions, as shown in Fig. 1.

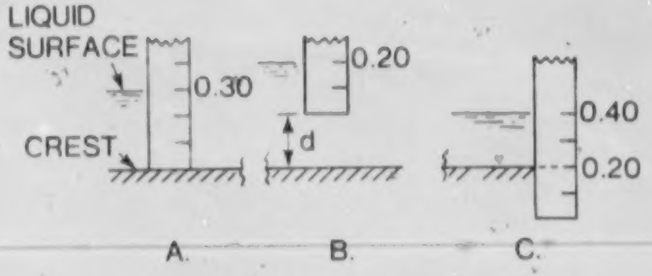

FIG. 1 - STAFF GAGE SETTINGS

TRAINING GUIDE NOTE		REFERENCES/RESOURCES

C.1 (cont.)

In "A", the bottom of the gage is set at crest elevation. The intersection of the liquid surface with the gage gives a direct reading of the head. Here, the head is 0.30 ft.

In "B" the bottom of the gage is set some distance "d" above crest elevation. To obtain the head, "d" must be added to the gage reading. For example, if "d" in the Figure equals 0.25 ft., then the head is 0.25 + 0.20, equals 0.45 ft.

In"C" the bottom of the gage is set some distance (say 0.20 ft) below crest elevation. This must be subtracted from the gage reading to obtain the head. Thus, 0.40-0.20 = 0.20 ft., which is the head.

Case II - Initial gage mark other than 0.00 ft.

The mark at which the gage divisions start must be taken into account in determining the head. For example, if a gage section starting at 3.33 ft. instead of 0.00 ft. is used, the calculations are as follows for the three conditions shown in Fig. 2 (which correspond to those of Fig. 1):

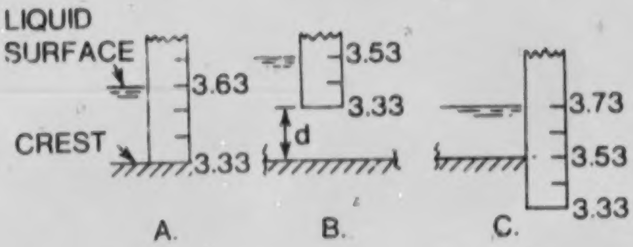

FIG. 2 - STAFF GAGE SETTINGS

In "A", head = 3.63-3.33 = 0.30 ft.
In "B", assuming that "d" = 0.25 ft., head = (3.53 + 0.25)-3.33 = 0.45 ft.
In "C", head = 3.73-3.53 = 0.20 ft.

344

TABLE 1

GCH

FREE FLOW DISCHARGE TABLE FOR PARSHALL MEASURING FLUME

HWH

SEC-FT X .646317 = M.G.D.
M.G.D. X 1.54723 = SEC-FT

SEC-FT X 448.831 = G.P.M.
G.P.M. X .002228 = SEC-FT

THROAT WIDTH

HEAD		6"			9"			12"			18"			24"		
FT.	IN.	SEC-FT	G.P.M.	M.G.D.	SEC-FT	G.P.M.	M.G.D.	SEC-FT	G.P.M.	M.G.D.	SEC-FT	G.P.M.	M.G.D.	SEC-FT	G.P.M.	M.G.D.
0.10	1 3/16	0.05	22.4	0.032	0.09	40.4	.0580									
0.11	1 5/16	0.06	26.9	0.039	0.10	44.9	.0650									
0.12	1 7/16	0.07	31.4	0.045	0.12	53.8	.0775									
0.13	1 9/16	0.08	35.9	0.052	0.14	62.8	.0905									
0.14	1 11/16	0.09	40.4	0.058	0.15	67.3	.0970									
0.15	1 13/16	0.10	44.9	0.065	0.17	76.3	.1100									
0.16	1 15/16	0.11	49.4	0.071	0.19	85.2	.1230									
0.17	2 1/16	0.12	53.8	0.077	0.20	89.8	.1290									
0.18	2 3/16	0.14	62.8	0.090	0.22	98.7	.1420									
0.19	2 1/4	0.15	67.3	0.097	0.24	107.7	.1550									
0.20	2 3/8	0.16	71.6	0.103	0.26	116.9	.1680	0.35	157.1	0.226	0.51	229.0	0.330	0.66	296.3	0.426
0.21	2 1/2	0.18	80.7	0.116	0.28	125.7	.1810	0.37	166.1	0.239	0.55	247.0	0.355	0.71	318.7	0.458
0.22	2 5/8	0.19	85.2	0.123	0.30	134.6	.1940	0.40	179.5	0.259	0.59	265.0	0.381	0.77	345.6	0.498
0.23	2 3/4	0.20	89.8	0.129	0.32	143.6	.2070	0.43	193.0	0.278	0.63	283.0	0.407	0.82	368.0	0.530
0.24	2 7/8	0.22	98.7	0.142	0.35	157.1	.2260	0.46	206.5	0.297	0.67	301.0	0.433	0.88	395.0	0.569
0.25	3	0.23	103.3	0.149	0.37	166.1	.2390	0.49	219.9	0.316	0.71	318.7	0.458	0.93	417.5	0.601
0.26	3 1/8	0.25	112.2	0.162	0.39	175.	.2520	0.51	229.0	0.330	0.76	341.1	0.491	0.99	444.3	0.640
0.27	3 1/4	0.26	116.9	0.168	0.41	184.0	.2650	0.54	242.3	0.349	0.80	359.0	0.517	1.05	471.3	0.679
0.28	3 3/8	0.28	125.7	0.181	0.44	197.5	.2840	0.58	260.3	0.374	0.85	381.5	0.549	1.11	498.2	0.717
0.29	3 1/2	0.29	130.2	0.187	0.46	206.5	.2970	0.61	274.0	0.394	0.90	404.0	0.582	1.18	530.0	0.763
0.30	3 5/8	0.31	139.2	0.203	0.49	219.9	.3160	0.64	287.3	0.414	0.94	422.0	0.607	1.24	556.6	0.801
0.31	3 3/4	0.32	143.6	0.207	0.51	229.0	.3300	0.68	305.2	0.439	0.99	444.3	0.640	1.30	583.4	0.840
0.32	3 13/16	0.34	152.6	0.220	0.54	242.3	.3490	0.71	318.7	0.458	1.04	466.8	0.672	1.37	615.0	0.886
0.33	3 15/16	0.36	161.5	0.233	0.56	251.3	.3620	0.74	332.0	0.478	1.09	489.0	0.704	1.44	646.0	0.930
0.34	4 1/16	0.38	170.5	0.246	0.59	265.0	.3810	0.77	345.6	0.498	1.14	512.0	0.737	1.50	673.2	0.969
0.35	4 3/16	0.39	175.1	0.252	0.62	278.0	.4010	0.80	359.0	0.517	1.19	534.0	0.769	1.57	705.0	1.015
0.36	4 5/16	0.41	184.0	0.265	0.64	287.3	.4140	0.84	377.0	0.543	1.25	561.0	0.808	1.64	736.0	1.060
0.37	4 7/16	0.43	193.0	0.278	0.67	301.0	.4330	0.88	395.0	0.569	1.30	583.4	0.840	1.72	772.0	1.111
0.38	4 9/16	0.45	201.9	0.291	0.70	314.0	.4520	0.92	412.9	0.594	1.36	610.0	0.879	1.79	803.4	1.156
0.39	4 11/16	0.47	210.9	0.304	0.73	327.6	.4720	0.95	426.0	0.614	1.41	633.0	0.911	1.86	835.0	1.202
0.40	4 13/16	0.48	215.5	0.310	0.76	341.1	.4912	0.99	444.3	0.640	1.47	659.0	0.950	1.93	866.0	1.247
0.41	4 15/16	0.50	224.4	0.323	0.78	350.0	.5040	1.03	462.0	0.666	1.53	686.0	0.989	2.01	902.0	1.299
0.42	5 1/16	0.52	233.3	0.336	0.81	363.5	.5240	1.07	480.0	0.692	1.58	709.0	1.021	2.09	938.0	1.350
0.43	5 3/16	0.54	242.3	0.349	0.84	377.0	.5430	1.11	498.1	0.717	1.64	736.0	1.060	2.16	969.0	1.396
0.44	5 1/4	0.56	251.3	0.362	0.87	390.4	.5620	1.15	516.0	0.743	1.70	763.0	1.098	2.24	1005	1.447
0.45	5 3/8	0.58	260.3	0.375	0.90	404.0	.5820	1.19	534.0	0.769	1.76	790.0	1.137	2.32	1041	1.499
0.46	5 1/2	0.61	274.0	0.394	0.94	422.0	.6075	1.23	552.0	0.795	1.82	816.0	1.176	2.40	1077	1.551
0.47	5 5/8	0.63	283.0	0.407	0.97	435.0	.6270	1.27	570.0	0.821	1.88	843.0	1.215	2.48	1113	1.602
0.48	5 3/4	0.65	292.0	0.420	1.00	448.8	.6460	1.31	588.0	0.847	1.94	870.0	1.254	2.57	1153	1.661
0.49	5 7/8	0.67	301.0	0.433	1.03	462.0	.6660	1.35	606.0	0.872	2.00	898.0	1.293	2.65	1189	1.712
0.50	6	0.69	310.0	0.446	1.06	475.7	.6850	1.39	624.0	0.898	2.06	925.0	1.331	2.73	1225	1.764
0.51	6 1/8	0.71	318.7	0.459	1.10	493.7	.7109	1.44	646.0	0.930	2.13	956.0	1.376	2.82	1266	1.822
0.52	6 1/4	0.73	327.6	0.472	1.13	507.0	.7300	1.48	664.0	0.956	2.19	983.0	1.415	2.90	1301	1.875
0.53	6 3/8	0.76	341.1	0.491	1.16	521.0	.7500	1.52	682.0	0.982	2.25	1010	1.454	2.99	1342	1.932
0.54	6 1/2	0.78	350.0	0.504	1.20	538.0	.7760	1.57	705.0	1.015	2.32	1041	1.499	3.08	1382	1.990
0.55	6 5/8	0.80	359.0	0.517	1.23	552.0	.7950	1.62	727.0	1.047	2.39	1073	1.545	3.17	1422	2.049
0.56	6 3/4	0.82	368.0	0.530	1.26	565.5	.8140	1.66	745.0	1.073	2.45	1099	1.583	3.26	1463	2.107
0.57	6 13/16	0.85	381.5	0.549	1.30	583.4	.8400	1.70	763.0	1.098	2.52	1131	1.628	3.35	1504	2.165
0.58	6 15/16	0.87	390.4	0.562	1.33	597.0	.8600	1.75	786.0	1.131	2.59	1163	1.674	3.44	1544	2.224
0.59	7 1/16	0.89	399.5	0.575	1.37	615.0	.8860	1.80	808.0	1.163	2.66	1194	1.719	3.53	1585	2.282
0.60	7 3/16	0.92	412.9	0.595	1.40	628.0	.9050	1.84	826.0	1.189	2.73	1225	1.764	3.62	1625	2.340
0.61	7 5/16	0.94	422.0	0.608	1.44	646.0	.9300	1.88	843.0	1.215	2.80	1257	1.809	3.72	1670	2.404
0.62	7 7/16	0.97	435.0	0.627	1.48	664.0	.9560	1.93	866.0	1.247	2.87	1290	1.855	3.81	1710	2.463
0.63	7 9/16	0.99	444.3	0.640	1.51	678.0	.9760	1.98	889.0	1.280	2.95	1324	1.907	3.91	1755	2.527
0.64	7 11/16	1.02	457.8	0.659	1.55	696.0	1.002	2.03	911.0	1.312	3.02	1355	1.952	4.01	1800	2.592
0.65	7 13/16	1.04	466.8	0.672	1.59	714.0	1.028	2.08	934.0	1.345	3.09	1387	1.997	4.11	1845	2.656
0.66	7 15/16	1.07	480.0	0.692	1.63	732.0	1.054	2.13	956.0	1.376	3.17	1422	2.049	4.20	1885	2.714
0.67	8 1/16	1.10	493.7	0.711	1.66	745.0	1.073	2.18	978.0	1.409	3.24	1454	2.094	4.30	1930	2.780
0.68	8 3/16	1.12	503.0	0.724	1.70	763.0	1.098	2.23	1001	1.441	3.31	1486	2.140	4.40	1975	2.844
0.69	8 1/4	1.15	516.0	0.743	1.74	781.0	1.125	2.28	1024	1.474	3.39	1522	2.191	4.50	2020	2.908
0.70	8 3/8	1.17	525.1	0.756	1.78	799.0	1.151	2.33	1046	1.506	3.46	1553	2.236	4.60	2065	2.973

HEAD		6"			9"			12"			18"			24"		
FT	IN	SEC-FT	G.P.M.	M.G.D.	SEC-FT	G.P.M.	M.G.D.	SEC-FT	G.P.M.	M.G.D.	SEC-FT	G.P.M.	M.G.D.	SEC-FT	G.P.M.	M.G.D.
0.71	8 1/2	1.20	538	0.776	1.82	816	1.176	2.38	1068	1.538	3.54	1590	2.288	4.70	2111	3.038
0.72	8 5/8	1.23	552	0.795	1.86	835	1.202	2.43	1091	1.571	3.62	1625	2.340	4.81	2159	3.110
0.73	8 3/4	1.26	566	0.814	1.90	853	1.228	2.48	1113	1.602	3.69	1656	2.385	4.91	2204	3.174
0.74	8 7/8	1.28	575	0.827	1.94	870	1.254	2.53	1136	1.635	3.77	1692	2.437	5.02	2253	3.245
0.75	9	1.31	588	0.847	1.98	889	1.280	2.58	1158	1.667	3.85	1728	2.488	5.12	2300	3.310
0.76	9 1/8	1.34	601	0.866	2.02	907	1.306	2.63	1182	1.700	3.93	1764	2.540	5.23	2347	3.380
0.77	9 1/4	1.36	610	0.879	2.06	925	1.331	2.68	1203	1.732	4.01	1800	2.592	5.34	2397	3.451
0.78	9 3/8	1.39	624	0.898	2.10	943	1.357	2.74	1230	1.771	4.09	1836	2.643	5.44	2442	3.516
0.79	9 1/2	1.42	637	0.918	2.14	960	1.383	2.80	1257	1.809	4.17	1872	2.695	5.55	2491	3.587
0.80	9 5/8	1.45	651	0.937	2.18	978	1.409	2.85	1280	1.842	4.26	1912	2.753	5.66	2540	3.658
0.81	9 3/4	1.48	664	0.957	2.22	996	1.435	2.90	1301	1.875	4.34	1948	2.805	5.77	2597	3.730
0.82	9 13/16	1.50	673	0.970	2.27	1019	1.467	2.96	1329	1.913	4.42	1984	2.857	5.88	2639	3.800
0.83	9 15/16	1.53	686	0.989	2.31	1036	1.493	3.02	1355	1.952	4.50	2020	2.908	6.00	2692	3.878
0.84	10 1/16	1.56	700	1.008	2.35	1054	1.518	3.07	1377	1.984	4.59	2060	2.967	6.11	2742	3.950
0.85	10 3/16	1.59	714	1.028	2.39	1073	1.545	3.12	1400	2.017	4.67	2096	3.018	6.22	2791	4.020
0.86	10 5/16	1.62	727	1.047	2.44	1095	1.577	3.18	1427	2.055	4.76	2136	3.076	6.33	2841	4.091
0.87	10 7/16	1.65	741	1.066	2.48	1113	1.602	3.24	1454	2.094	4.84	2171	3.128	6.44	2891	4.162
0.88	10 9/16	1.68	754	1.085	2.52	1131	1.628	3.29	1476	2.126	4.93	2212	3.186	6.56	2944	4.240
0.89	10 11/16	1.71	768	1.105	2.57	1153	1.661	3.35	1504	2.165	5.01	2248	3.238	6.68	2998	4.318
0.90	10 13/16	1.74	781	1.125	2.61	1171	1.686	3.41	1530	2.203	5.10	2289	3.296	6.80	3052	4.394
0.91	10 15/16	1.77	794	1.144	2.66	1194	1.720	3.46	1553	2.236	5.19	2329	3.354	6.92	3105	4.473
0.92	11 1/16	1.81	812	1.169	2.70	1212	1.745	3.52	1580	2.275	5.28	2370	3.413	7.03	3155	4.543
0.93	11 3/16	1.84	826	1.189	2.75	1234	1.777	3.58	1606	2.313	5.37	2410	3.470	7.15	3209	4.621
0.94	11 1/4	1.87	839	1.208	2.79	1252	1.803	3.64	1633	2.352	5.46	2450	3.529	7.27	3263	4.698
0.95	11 3/8	1.90	853	1.228	2.84	1274	1.835	3.70	1661	2.391	5.55	2491	3.587	7.39	3316	4.777
0.96	11 1/2	1.93	866	1.247	2.88	1292	1.862	3.76	1687	2.430	5.64	2531	3.645	7.51	3370	4.853
0.97	11 5/8	1.97	884	1.273	2.93	1315	1.894	3.82	1714	2.469	5.73	2572	3.703	7.63	3425	4.930
0.98	11 3/4	2.00	898	1.293	2.98	1337	1.926	3.88	1741	2.510	5.82	2612	3.762	7.75	3478	5.009
0.99	11 7/8	2.03	911	1.312	3.02	1355	1.952	3.94	1768	2.547	5.91	2653	3.820	7.88	3537	5.093
1.00	12	2.06	925	1.331	3.07	1377	1.984	4.00	1795	2.585	6.00	2692	3.878	8.00	3590	5.170
1.01	12 1/8	2.09	938	1.350	3.12	1400	2.017	4.06	1822	2.624	6.09	2733	3.936	8.12	3645	5.248
1.02	12 1/4	2.12	952	1.370	3.17	1422	2.049	4.12	1849	2.662	6.19	2778	4.000	8.25	3703	5.332
1.03	12 3/8	2.16	969	1.396	3.21	1440	2.074	4.18	1876	2.701	6.28	2818	4.059	8.38	3761	5.416
1.04	12 1/2	2.19	983	1.415	3.26	1463	2.107	4.25	1908	2.747	6.37	2859	4.117	8.50	3815	5.494
1.05	12 5/8	2.22	996	1.435	3.31	1486	2.140	4.31	1934	2.786	6.47	2904	4.182	8.63	3873	5.578
1.06	12 3/4	2.26	1014	1.460	3.36	1508	2.172	4.37	1961	2.824	6.56	2944	4.240	8.76	3932	5.662
1.07	12 13/16	2.29	1027	1.480	3.40	1526	2.197	4.43	1988	2.863	6.66	2989	4.304	8.88	3986	5.740
1.08	12 15/16	2.32	1041	1.500	3.45	1548	2.230	4.50	2020	2.908	6.75	3030	4.362	9.01	4044	5.823
1.09	13 1/16	2.36	1060	1.525	3.50	1571	2.262	4.56	2047	2.947	6.85	3075	4.427	9.14	4102	5.907
1.10	13 3/16	2.40	1077	1.551	3.55	1594	2.295	4.62	2074	2.986	6.95	3119	4.492	9.27	4161	5.991
1.11	13 5/16	2.43	1091	1.571	3.60	1616	2.327	4.68	2101	3.025	7.04	3160	4.550	9.40	4220	6.075
1.12	13 7/16	2.46	1104	1.590	3.65	1638	2.360	4.75	2132	3.070	7.14	3205	4.614	9.54	4282	6.165
1.13	13 9/16	2.50	1122	1.616	3.70	1661	2.391	4.82	2164	3.115	7.24	3250	4.680	9.67	4340	6.250
1.14	13 11/16	2.53	1136	1.635	3.75	1683	2.424	4.88	2190	3.154	7.34	3294	4.744	9.80	4399	6.334
1.15	13 13/16	2.57	1153	1.661	3.80	1706	2.456	4.94	2217	3.193	7.44	3339	4.810	9.94	4461	6.424
1.16	13 15/16	2.60	1167	1.681	3.85	1728	2.488	5.01	2248	3.238	7.54	3384	4.873	10.1	4578	6.528
1.17	14 1/16	2.64	1185	1.706	3.90	1750	2.521	5.08	2280	3.283	7.64	3429	4.940	10.2	4581	6.593
1.18	14 3/16	2.68	1203	1.732	3.95	1773	2.553	5.15	2311	3.329	7.74	3474	5.003	10.3	4623	6.657
1.19	14 1/4	2.71	1217	1.752	4.01	1800	2.592	5.21	2338	3.367	7.84	3519	5.067	10.5	4713	6.786
1.20	14 3/8	2.75	1234	1.777	4.06	1822	2.624	5.28	2370	3.413	7.94	3564	5.132	10.6	4760	6.851
1.21	14 1/2	2.78	1248	1.797	4.11	1845	2.656	5.34	2397	3.451	8.05	3613	5.203	10.8	4850	6.980
1.22	14 5/8	2.82	1266	1.823	4.16	1867	2.689	5.41	2428	3.497	8.15	3658	5.267	10.9	4892	7.045
1.23	14 3/4	2.86	1284	1.848	4.21	1890	2.721	5.48	2460	3.542	8.25	3703	5.332	11.0	4940	7.110
1.24	14 7/8	2.89	1297	1.867	4.27	1917	2.760	5.55	2491	3.587	8.36	3752	5.403	11.2	5027	7.240
1.25	15	2.93	1315	1.894	4.32	1939	2.793	5.62	2522	3.632	8.46	3800	5.468	11.3	5072	7.304
1.26	15 1/8	2.97	1333	1.920	4.37	1961	2.824	5.69	2554	3.678	8.56	3842	5.533	11.5	5162	7.433
1.27	15 1/4	3.01	1350	1.945	4.43	1988	2.863	5.76	2585	3.723	8.67	3892	5.603	11.6	5210	7.497
1.28	15 3/8	3.04	1364	1.965	4.48	2011	2.896	5.82	2612	3.762	8.77	3936	5.668	11.7	5251	7.562
1.29	15 1/2	3.08	1382	1.991	4.53	2033	2.928	5.89	2644	3.807	8.88	3986	5.740	11.9	5341	7.691
1.30	15 5/8	3.12	1400	2.017	4.59	2060	2.967	5.96	2675	3.852	8.99	4035	5.810	12.0	5386	7.756
1.31	15 3/4	3.16	1418	2.042	4.64	2083	3.000	6.03	2710	3.900	9.09	4080	5.875	12.2	5476	7.885

WATER & SEWAGE WORKS, REFERENCE AND DATA SECTION, 1954 P. R-277.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
C.2	With H_a determined, the flow can be obtained from a Table such as that shown in Table 1. (This shows the head-discharge relationships for flumes ranging in size from 6 to 24 inches. Similar tables for larger and smaller flumes will be found in References 1, 2, and 3).	4. Water and Sewage Works, Reference & Data Section, 1954, p. R-277

The flow is obtained from this Table as follows:

1. Go vertically downward in the column titled "Head" until you reach the value for the H_a measured. Note that values of Head in this column are given both in feet, and in inches corresponding to the foot values.
2. Proceed horizontally to the right until you reach the columns for the throat width of flume in use.
3. Read the flow in the units to be reported. The flow is given in three units in this Table: sec.-ft. (cubic feet per second); GPM (gallons per minute), and MGD (million gallons per day). Example:

   For a 12" flume, with H_a = 0.86 ft.

   1. Locate 0.86 ft. in the "Head" column.
   2. Go over horizontally to the right to the columns under the "12" throat width.
   3. Read flow: 3.18 sec.-ft., or
      1427 GPM, or
      2.06 MGD

D.1 A float gage is shown in Fig. 3, installed in a stilling well for measurement of H_a. To illustrate the calculation of H_a it is assumed that the floor of the stilling well is at the same elevation as the crest of the flume, and that the liquid is flowing in the flume with a depth of one foot. The float will, of course, be resting on the surface of the liquid in the well, and it is also assumed that for the condition illustrated the tape division opposite the float index reads 8-1/2 feet.

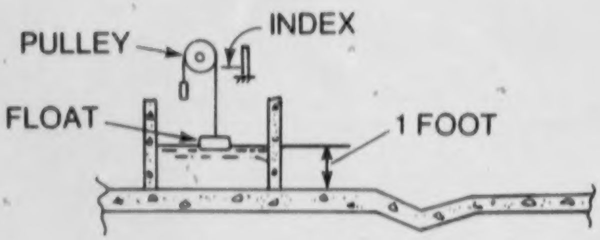

FIG.3 - FLOAT GAGE INSTALLATION

With the specific relations established for this one condition, the gage can now be "zeroed" so that H_a can be obtained for any other condition, as follows:

(a) A reading of 8-1/2 feet on the tape corresponds to an H_a of one foot. Consequently, a reading of 7-1/2 feet on the tape corresponds to an H_a of zero feet, or to the crest elevation.

(b) Therefore H_a can be obtained for any depth of flow by subtracting 7-1/2 feet from the observed tape division opposite the index.

The following points should be noted in connection with this procedure:

(a) If the elevation of the index is changed, the gage must be re-zeroed.

TRAINING GUIDE NOTE		REFERENCES/RESOURCES
D.1 (cont.)	(b) If the position of the tape on the pulley is changed, the gage must be re-zeroed. (c) The tape must be installed so that the numerical value of the tape reading increases as the depth of flow increases. H_b can be obtained with the float gage in the same manner as described above for H_a.	
E.3.2	Discharge through the flume is not reduced from the free-flow value until the percent submergence equals or exceeds the following values: 60% for 6-inch and 9-inch flumes 70% for 1 foot to 8-foot flumes When the submergence reaches these values, a corrected flow must be calculated in the following manner: For 6-inch and 9-inch Flumes The corrected flow can be obtained directly from Fig. 4 for a 6-inch flume, and from Fig. 5 for a 9-inch flume. Example: For a 6-inch flume, $H_a = 1.0$ ft., $H_b = 0.8$ ft. $\% \text{ Submergence} = \frac{0.8}{1.0} \times 100 = 80\%$ Refer to Fig. 4. On "Percent of submergence" scale on left-hand side, go up to the "80" value. Move to the right along the "80" line to where it intersects the "H_a = 1.0 feet" curve. Drop vertically from the point of intersection to the "Discharge, Second-feet" scale along the bottom of the chart. Read 1.7 - this is the discharge in cubic feet per second through the flume. Convert flow to other units if desired. Exactly the same procedure would be followed for a 9-inch flume, using Fig. 5.	Ref. 1

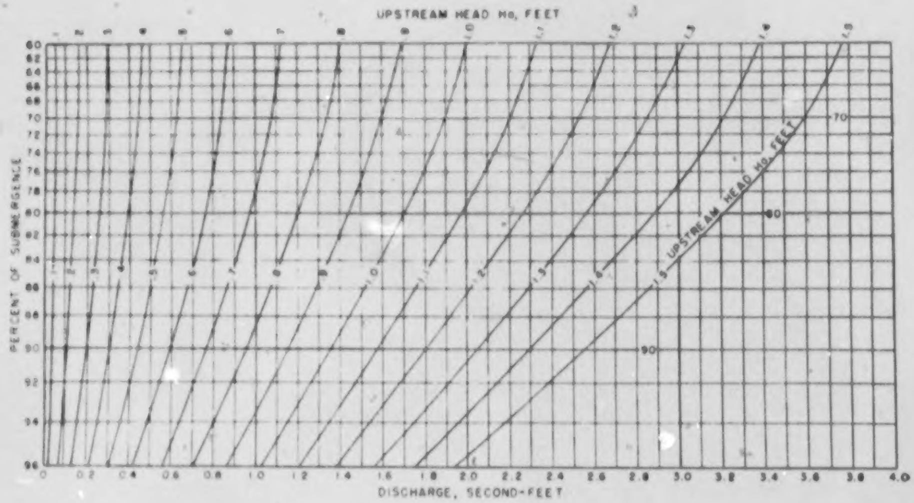

FIG. 4—Diagram for determining rate of submerged flow for a 6-inch Parshall flume. 103-D-897. (Courtesy U.S. Soil Conservation Service.)

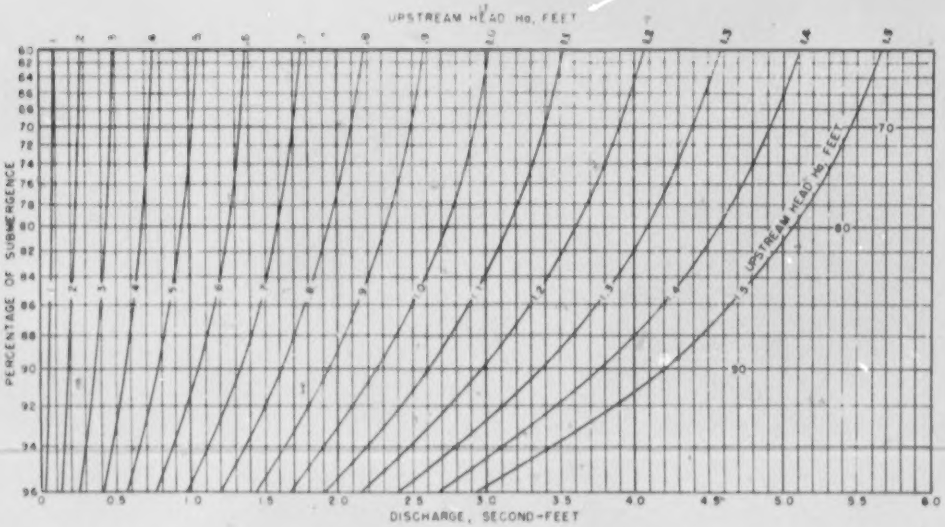

FIG. 5—Diagram for determining rate of submerged flow for a 9-inch Parshall flume. 103-D-898. (Courtesy U.S. Soil Conservation Service.)

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E.3.2
(cont.)

For Flumes 1 foot to 8 feet wide

Use Fig. 6. This provides a correction factor to be applied to the discharge obtained using H_a and Table 1, the free-flow discharge table. For flumes larger than 1 foot a second correction, using a "multiplying factor" is necessary. Example 1.

For a 1-foot flume,

$H_b = 0.8$ ft., $H_a = 1.0$ ft.

$$\text{Submergence} = \frac{0.8}{1.0} \times 100 = 80\%$$

Refer to Fig. 6. On "Upstream Head H_a" scale at left-hand side, go up to 1.0 ft. Move to the right along the "1.0 ft." line to where it intersects the "80% Submergence" curve. Drop vertically from the point of intersection to the "Correction, second-feet" scale at the bottom of the chart. Read "0.35 sec.-ft."

Refer to Table 1. For a 12-inch flume with $H_a = 1.0$ ft., discharge is 4.00 sec.-ft. But the actual discharge will be less than this, since submergence exceeds 70%. To get actual discharge, subtract correction obtained from Fig. 6. Then the discharge is 4.00-0.35 = 3.65 sec.-ft.

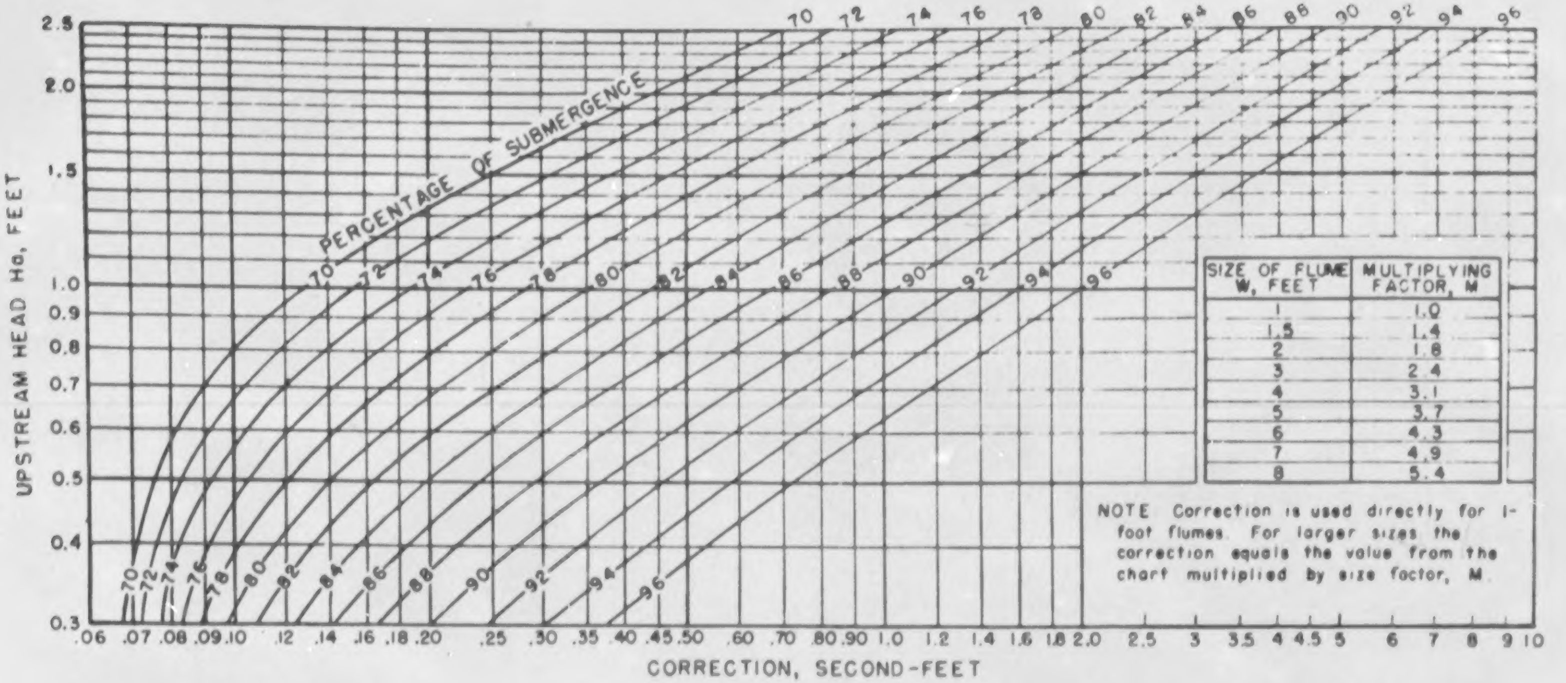
Note that "Multiplying Factor" is 1.0, so the correction factor obtained from Fig. 6 is used directly.

Example 2
For a 24-inch flume,

$H_b = 1.23$ ft., $H_a = 1.30$ ft.

$$\text{Submergence} = \frac{1.23}{1.30} \times 100 = 95\%$$

Refer to Fig. 6. On left-hand scale go up to 1.30 ft., which is the Upstream Head H_a.



SIZE OF FLUME W, FEET	MULTIPLYING FACTOR, M
1	1.0
1.5	1.4
2	1.8
3	2.4
4	3.1
5	3.7
6	4.3
7	4.9
8	5.4

NOTE Correction is used directly for 1-foot flumes. For larger sizes the correction equals the value from the chart multiplied by size factor, M.

FIG. 6—Diagram for determining correction to be subtracted from free-discharge flow to obtain rate of submerged flow through Parshall flumes 1 to 8 feet wide. 103–D–875. (Courtesy U.S. Soil Conservation Service.)

EDUCATIONAL CONCEPTS - MATHEMATICS

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
E.3.2 (cont.)	Proceed horizontally to the right along the "1.30 ft." line. The point of intersection of this line with the "95% Submergence" curve is to be located. Since no curve is drawn on Fig. 6 for this value of submergence, mentally locate a point on the "1.30 ft." line which is midway between the "94% submergence" and the "96% submergence" curves. Drop vertically from this point to intersect the "Correction, second-feet" scale at the bottom of the chart. Read "2.7". From the Table at right side of chart on Fig. 6, read the "Multiplying Factor" for a 24-inch flume. This factor is 1.8. Multiply. 2.7 x 1.8 = 4.9 sec.-ft. This is the correction factor to be used in this case. From Table I obtain free-flow discharge of 12.0 sec.-ft. for a 2-foot flume with H_a = 1.30 ft. Subtract correction factor from this free-flow value to obtain discharge with this degree of submergence. Discharge = 12.0 - 4.9 = 7.1 sec.-ft.	

B.1.1 The Parshall Flume is intended for use as an in-line structure in an open channel where reasonably smooth flow, uniformly distributed across the cross-section, is the normal condition.

A good degree of accuracy cannot be maintained if poor approach conditions exist in the approach channel. Experience has shown that Parshall Flumes should not be placed at right angles to flowing streams unless the flow is effectively straightened and uniformly redistributed before it enters the flume. Surges and waves of any appreciable size should be eliminated.

The liquid should enter the converging section reasonably well distributed across the entrance width, and the flowlines should be essentially parallel to the flume centerline. Flow at the flume entrance should be free of "white" water and free from turbulence in the form of visible surface boils. Only then can the flume measure flow as intended.

B.1.3 The velocity of flow through the flume will generally be sufficiently great to virtually eliminate any deposition of sediment within the structure. If any such build-up is observed, however, it should be eliminated. Deposits should also be removed from the channel upstream and downstream of the flume.

B.1.4 The flow condition can be determined from measurements of H_a and H_b. Generally, however, these heads do not have to be measured--the condition of flow through the flume can usually be determined by visual observation.

Three flow conditions through the flume are shown in Fig. 7.

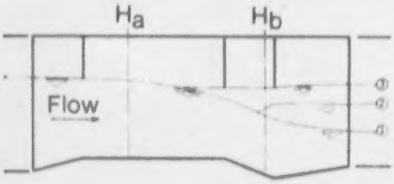


FIG. 7 - FLOW CONDITIONS

TRAINING GUIDE NOTE

REFERENCES/RESOURCES

B.1.4 (cont.) In flow condition 2, there is a drop in the elevation of the liquid surface, followed by an abrupt rise in the throat. This phenomenon is referred to as a hydraulic jump or standing wave. When the hydraulic jump is present, free-flow conditions exist.

In flow condition 1, there is a substantial and smooth drop in the elevation of the liquid surface as it passes through the throat and the diverging section of the flume. Free-flow conditions exist. A hydraulic jump will be observed downstream of the flume.

Flow condition 3 illustrates the configuration of the liquid surface for submerged-flow conditions. Sometimes a series of waves or ripples will be noted in the transition area between the upstream and downstream liquid elevations. These also indicate that submerged-flow conditions exist.

Flumes used in treatment plants are selected to operate under free-flow conditions over the range of flows handled at the plant. The existence of a submerged-flow condition would therefore be most unusual, and might be due either to the flume being too small, or to some obstruction in the channel downstream of the flume which is raising the water level. In any case, it is important to determine the reason for a submerged flow condition, and take the appropriate steps to return the flume to free-flow operation.

B.1.5 For a stilling well to function properly, the opening or pipe between the well and the flume must be kept free of deposits or materials which would interfere with the free movement of liquid. This should be checked occasionally, and any such interferences removed by flushing with clean water, or by some other suitable procedure. Deposits or floating materials in the well should also be removed to maintain accurate head measurement.

A.3

Two drawings of a Parshall Flume are shown in Fig. 8. The top drawing shows the appearance of the flume when viewed from above. The drawing labeled "Section L-L" is the way the flume looks when viewed from the side, along the line marked "L-L" in the top drawing.

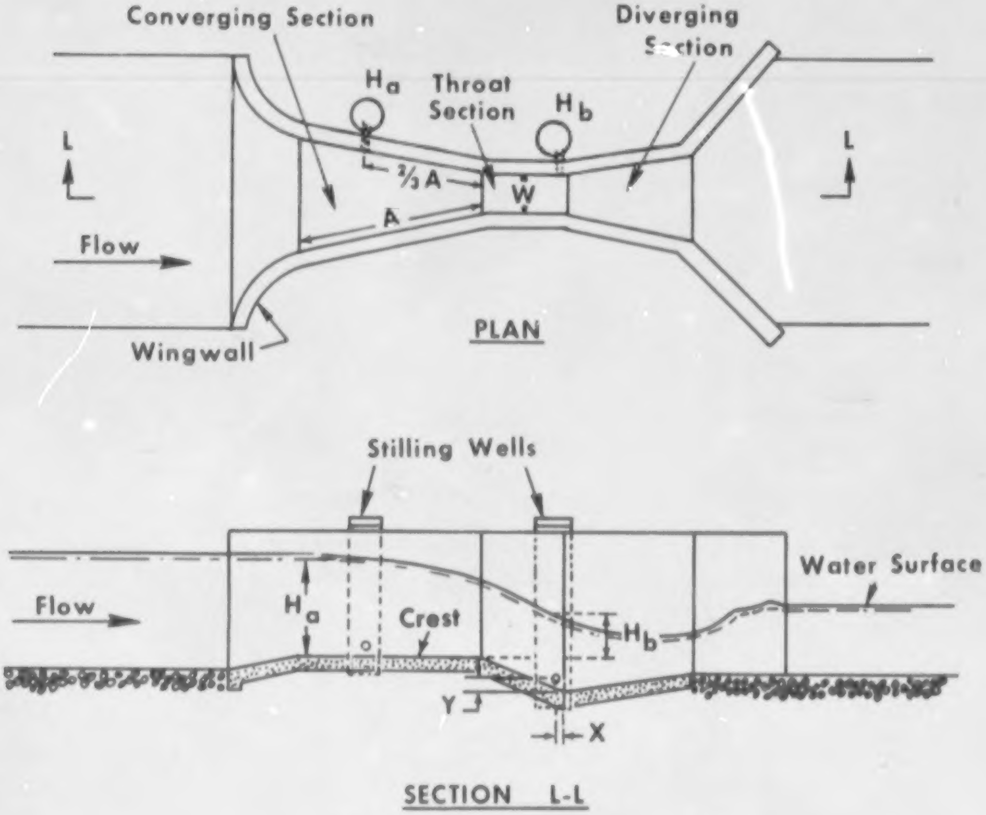

Converging Section
Diverging Section
H_a
Throat Section
H_b
L
L
⅔ A
W
A
Flow
Wingwall
PLAN
Stilling Wells
Water Surface
Flow
H_a
Crest
H_b
Y
X
SECTION L-L

Fig. 8. PARSHALL FLUME

The flume structure proper consists of three sections:

1. A converging section
2. A throat section
3. A diverging section

(continued)

A.3 (cont'd.) Wing walls are shown in Fig. 8 immediately upstream and downstream of the flume. In situations where the channel in which the flume is located is wider than the beginning of the converging section and the end of the diverging section of the flume, these wing walls provide a gradual transition in width of the flowing liquid. Their function is to ensure proper approach and "getaway" conditions to avoid measurement difficulties. Curved wingwalls are preferred over straight 45° walls, although any arrangement that achieves uniformity and smoothness in the flow is acceptable.

Flumes can be made of concrete, galvanized steel, plastic, wood, or other suitable material. Flume must be built to specific dimensions, for which tables are available (page 47, Ref. 2), and close tolerances for accurate performance. The floor of the converging section (flume crest) must be level if the flume is to operate properly.

A.4 Throat Width
Distance between the walls of the throat section.

Flume Size
Flumes are designated as to size by the throat width, as a "6-inch flume," a "10-foot flume," etc.

Flume Crest
Floor of the converging section. Sometimes indicated as the junction point of the floor of the converging section with the throat section.

Crest Elevation
Elevation of the floor of the converging section.

Upstream Head (Ha)
Depth of liquid in the converging section, measured at a point two-thirds of the section length upstream from the throat (see Fig. 8).

Downstream Head (Hb)
Depth of liquid over the flume crest, measured at a specific point in the throat section. For flumes considered in this guide the point of measurement is 2 inches upstream of the beginning of the diverging section. Because of turbulence in the throat section, it is often impossible to determine the head accurately with a staff gage, and a stilling well should be provided. The connection between the throat and

(continued)

A.4 (cont'd.) stilling well must be located two inches upstream from the low point in the floor (dimension X. Fig. 8) and 3 inches above it (dimension Y, Fig. 8).

Stilling Well (Float Well)
A chamber connected through a small opening to the liquid flowing in an open channel. Waves and surges occurring in the flowing liquid will not appear in the well. Liquid level in the well will follow all the steady fluctuations of the flowing liquid. The well must have a bottom and be practically water tight except for the liquid inlet.

Free Flow
A flow condition in which liquid passing over the crest of the flume is not impeded or slowed by downstream conditions.

Submerged Flow
In most installations, when the discharge through the flume is increased above a certain critical value the resistence to flow in the downstream channel becomes large enough to reduce the velocity, increase the flow depth and cause a back water effect at the flume, in which the flow is retarded and discharge reduced.

Submergence
The ratio $\frac{H_b}{H_a}$, usually expressed as a percentage.

A.5 The Parshall Flume is a specially-shaped flow section, so constructed and installed that the rate of flow through it depends only on its size (throat width), and the depth of liquid over the crest. Discharge through the flume can occur for two conditions of flow:

1. Free Flow, in which the discharge depends only on the upstream head H_a. When free-flow conditions exist, discharge through the flume can be obtained by measuring the upstream head only.
2. Submerged Flow, in which the discharge is reduced due to the effect of the depth of liquid downstream of the flume. In this case it is necessary to measure both the upstream head H_a and the downstream head H_b in order to obtain the discharge.

A.6 A staff gage (Fig. 9) is a graduated scale, usually installed vertically, for obtaining liquid depth, or head. An observer notes the scale division at which the liquid surface intersects the gage (gage height). The head and discharge can then be calculated.

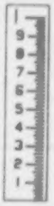
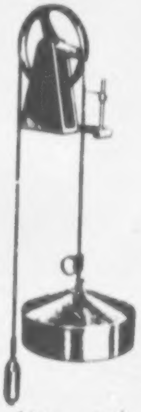


FIG. 9 - STAFF GAGE SECTION

Commercially-available gages are made of 18-gage metal coated with a substantial thickness of porcelain enamel. The face of the gage is white; numerals and graduations are black. Gages are available in several styles; in widths from 2-1/2 to 4 inches, in lengths from 1 to 5 feet. A gage divided in metric units is also commercially available.

A.7 A float gage (Fig. 10) is a means of continuously indicating liquid levels. It consists of a metal float, a pulley mounted on a standard, and a counterweight. A graduated stainless steel tape is attached to the float and connected at the other end to the counterweight. The float follows the rise and



(Fig. 10)

(continued)

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.7 (cont'd.)	fall of the liquid surface and the level can be read from the tape and a pointer or reference mark. Tapes are available in selected lengths, and are graduated either in feet, tenths and hundredths for English measurements, or meters, decimeters and centimeters for metric measurements. The float gage is used extensively as a reference gage in stilling wells to check the accuracy of automatic head or flow recording devices.	

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

MEASUREMENT OF FLOW IN AN OPEN CHANNEL BY SHARP-CRESTED WEIR

as applied in

WASTEWATER TREATMENT FACILITIES
and in
MONITORING OF EFFLUENT WASTEWATERS

Developed by the

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

EFFLUENT MONITORING PROCEDURE: Measurement of Flow in an Open Channel by Sharp-Crested Weir

This Procedure was developed by:

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Staff Engineer, then Chief, Water Quality Section, Denver Regional Office

Staff Engineer, then Regional Construction Grants Program Director, Denver Regional Office

Regional Construction Grants Program Director, Cincinnati Regional Office

Director, Colorado River Basin Water Quality Control Project, Denver, Colorado

Industrial Wastes Consultant, Technical Advisory and Investigations Branch, Cincinnati, Ohio

Participation in and Direction of numerous in-plant industrial waste surveys and stream studies in New York, Colorado, New Mexico, Maine, Utah

With National Training Center September 1969 to date.

EFFLUENT MONITORING PROCEDURE: Measurement of Flow in an Open Channel by Sharp-Crested Weir

1. Objective:

   The student will be able to make an acceptable measurement of flow rate in an open channel by means of a preinstalled sharp-crested weir and vertical staff gage or a float gage.

2. Brief Description of Procedure:

   The depth of liquid producing flow over a weir is measured. This measurement is used to obtain the rate of flow in the channel at the time the observation was made.

General Description of Equipment used in the Procedure:

1. A Weir over which the liquid flows.
2. Means for visually observing depth of liquid above the weir crest, such as a staff gage or a float gage.

EFFLUENT MONITORING PROCEDURE: Measurement of Flow in an Open Channel by Sharp-Crested Weir

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Basic Elements			
1. Units of Flow Measurement			II.A.1 (p. 9)
2. Description of Process			I.A.2 (p. 8)
3. Definitions			V.A.3 (p. 16)
4. Types of Weirs			V.A.4 (p. 16)
5. Staff Gage			V.A.5 (p. 18)
6. Float Gage			V.A.6 (p. 18)
B. Preparation for Measurement			
1. Physical Conditions	1. Inspect weir bulkhead.	1a. No leakage. 1b. Bulkhead vertical. 1c. Bulkhead perpendicular to direction of flow.	V.B.1.1 (p. 19)
	2. Inspect weir plate.	2a. Crest horizontal 2b. Crest at zero gage elevation 2c. No nicks or dents 2d. No clinging debris or build-up of grease, etc.	V.B.1.2 (p. 19)
	3. Inspect nappe.	3a. No submergence. 3b. Springs clear of downstream side of weir plate.	V.B.1.3 (p. 20)
	4. Inspect approach channel.	4a. No large submerged or floating objects. 4b. No excessive sediment deposits.	V.B.1.4 (p. 20)
	5. Allow flow to stabilize.	5a. Undisturbed flow condition.	V.B.1.5 (p. 21)

EFFLUENT MONITORING PROCEDURE: Measurement of Flow in an Open Channel
by Sharp-Crested Weir

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Flow Measurement Using Staff Gage			
1. Determination of Head	1. Read gage division at which liquid surface intersects gage.	1a. To nearest division. 1b. This reading should be made at a distance at least 2.5 H upstream of the weir.	II.C.1 (p. 10)
	2. Calculate head on weir.	2a. From staff gage reading. 2b. Should not be less than 0.2 feet.	
2. Determination of Flow Rate	1. Use appropriate weir table.		II.C.2 (p. 10)
D. Flow Measurement Using Float Gage			
1. Determination of Head	1. Read tape division opposite index on float gage.	1a. To nearest division 1b. This reading should be made at a distance at least 2.5 H upstream of the weir.	II.D.1 (p. 15)
	2. Calculate head on weir.	2a. From float gage reading 2b. Should not be less than 0.2 feet.	
2. Determination of Flow Rate	1. Use appropriate weir table.		II.C.2 (p. 15)

EFFLUENT MONITORING PROCEDURE: Measurement of Flow in an Open Channel by Sharp-Crested Weir

TRAINING GUIDE

SECTION	TOPIC
I*	Introduction
II*	Educational Concepts - Mathematics
III	Educational Concepts - Science
IV	Educational Concepts - Communication
V*	Field & Laboratory Equipment
VI	Field & Laboratory Reagents
VII	Field & Laboratory Analysis
VIII	Safety
IX	Records & Reports

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*Training guide materials are presented here under the headings marked *. These standardized headings are used throughout this series of procedures.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.2	Flow of a liquid in an open channel can often be conveniently and accurately measured by means of a sharp-crested weir installed in the channel. For a weir of specific size and shape with free-flow steady-state conditions and proper approach conditions, only one depth of liquid can exist upstream of the weir for a given flow. The flow is determined by measuring the vertical distance from the crest of the weir plate to the upstream liquid surface and then using a weir formula or weir table. The weir must have a standard shape and dimensions, and be installed so that the system performs in a standard manner.	1. Handbook of Hydraulics King, H.W., McGraw-Hill, NY, 3rd Ed. 1939 2. Water Measurement Manual, US Dept. Interior, Bur. Reclamation, Denver, CO, 2nd Ed. 1967 3. Stevens Water Resources Data Book. Leupold & Stevens, Inc., Box 688, Beaverton, Oregon, 97005. 2nd Ed. $4.00

TRAINING GUIDE NOTE | REFERENCES/RESOURCES

A.1

Flows - Units of Measurement

I. Flow, or Flow Rate, or Discharge.

All of these terms are commonly used to refer to the quantity of liquid passing a point in a certain time interval.

II. Quantity of liquid can be expressed in a number of ways. Common units are the gallon (Gal) and the cubic foot (cu. ft., ft.^{3}). To change from one of these measures to another, use the table below:

Multiply	by	To obtain
cu. ft.	7.5	Gal.
Gal.	0.134	cu. ft.

III. Flow is usually expressed in these units:

Gallons per minute (GPM)
Million gallons per day (MGD)
Cubic feet per second (cfs)

To change from one of these units to another, use this table:

Multiply	by	To obtain
cfs	0.646	MGD
MGD	1.55	cfs
cfs	448.8	GPM
GPM	0.0022	cfs
MGD	694.4	GPM
GPM	0.00144	MGD

IV. Flow data is needed to calculate the quantity of constituents discharged in a plant effluent. Formulas are--

$$\text{lb/day} = \text{MGD} \times \text{mg/l} \times 8.34$$
$$\text{Kg/day} = \text{MGD} \times \text{mg/l} \times 3.78$$

C.1 The head on the weir is calculated from the staff gage reading. Either of two conditions may exist, depending on zero gage elevation:

Case I - Zero gage elevation is at "0" on the gage

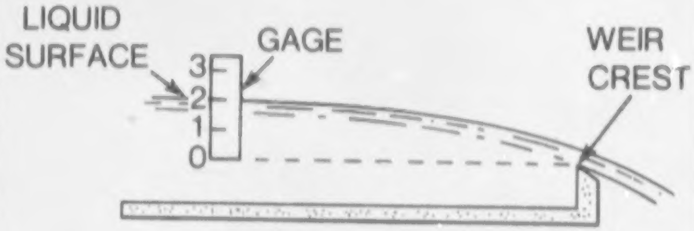

FIG. 1 - HEAD ON WEIR

The head on the weir corresponds to the gage division intersected by the surface of the liquid. In the above diagram H=2 feet.

Case II - Zero gage elevation is at some gage division other than "0"

The diagram below illustrates this case when the 1-foot division on the gage is at the same elevation as the weir crest.

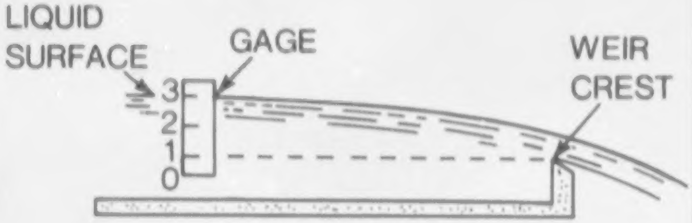

FIG. 2 - HEAD ON WEIR

Since the head on the weir is the difference between zero gage elevation and the gage division intersected by the liquid surface, H=3-1=2 feet.

C.2 Having determined the head on the weir, the flow rate can be obtained from a weir table. The proper table for the type of weir in use must be selected. The use of weir tables is shown below.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
C.2 (Cont'd)	Use of weir tables.	
	1. 90° V-notch weir - Table I This table lists flows corresponding to weir heads ranging from 0.10' to 2.09'. The flow for any head in this range can be read directly from the table. Example: For H=0.65' At intersection of values of 0.60 in left-hand head column and 0.05 in top column, read Q=0.852 cfs or 0.550 MGD	Ref. 3
	2. Standard Contracted Rectangular Weir - Table II Flows are given for various heads and for weirs having different crest lengths. Example: Weir crest=3' H=0.26' Read from table Q=1.30 cfs or 0.84 MGD	Ref. 3
	3. Standard Suppressed Rectangular Weir - Table III The format of this table differs from that of Table II, in that the flow is given per foot of weir crest length. Values obtained from the table must therefore be multiplied by the crest length of the weir to obtain the total flow. Example: Weir crest length=10' H=1.0' From table, Q=3.33 cfs or 2.15 MGD This is the flow per foot of weir length; therefore the total flow over the weir is Q=3.33 x 10=33.3 cfs or 21.5 MGD	Ref. 3

TABLE 1

DISCHARGE OF 90° V-NOTCH WEIRS

FORMULA CFS = 2.50 $H^{5/2}$ MGD = CFS x .646317

Head	.00		.01		.02		.03		.04		.05		.06		.07		.08		.09	
Ft.	CFS	MGD	CFS	MGD	CFS	MGD	CFS	MGD	CFS	MGD	CFS	MGD	CFS	MGD	CFS	MGD	CFS	MGD	CFS	MGD
0.1	.008	.005	.010	.006	.012	.008	.015	.010	.018	.012	.022	.014	.026	.017	.030	.019	.034	.022	.039	.025
0.2	.045	.029	.051	.033	.057	.037	.063	.041	.071	.046	.078	.050	.086	.056	.095	.061	.104	.067	.113	.073
0.3	.123	.079	.134	.087	.145	.094	.156	.101	.169	.109	.181	.117	.194	.125	.208	.134	.223	.144	.237	.153
0.4	.253	.164	.269	.174	.286	.185	.303	.196	.321	.207	.340	.220	.359	.232	.379	.245	.399	.258	.420	.271
0.5	.442	.286	.464	.300	.487	.315	.511	.330	.536	.346	.561	.363	.587	.379	.613	.396	.640	.414	.668	.432
0.6	.697	.450	.727	.470	.757	.489	.788	.509	.819	.529	.852	.551	.885	.572	.919	.594	.953	.616	.989	.639
0.7	1.03	.662	1.06	.686	1.10	.711	1.14	.726	1.18	.761	1.22	.787	1.26	.814	1.30	.841	1.34	.868	1.39	.896
0.8	1.43	.925	1.48	.954	1.52	.984	1.57	1.01	1.62	1.05	1.67	1.08	1.71	1.11	1.76	1.14	1.82	1.18	1.87	1.21
0.9	1.92	1.24	1.97	1.27	2.03	1.31	2.09	1.35	2.14	1.38	2.20	1.42	2.26	1.46	2.32	1.50	2.38	1.54	2.44	1.58
1.0	2.50	1.62	2.56	1.65	2.63	1.70	2.69	1.74	2.76	1.78	2.82	1.82	2.89	1.87	2.96	1.91	3.03	1.96	3.10	2.00
1.1	3.17	2.05	3.25	2.10	3.32	2.15	3.39	2.19	3.47	2.24	3.55	2.29	3.62	2.34	3.70	2.39	3.78	2.44	3.86	2.49
1.2	3.94	2.55	4.03	2.60	4.11	2.66	4.19	2.71	4.28	2.77	4.37	2.82	4.46	2.88	4.54	2.93	4.63	2.99	4.73	3.06
1.3	4.82	3.12	4.91	3.17	5.00	3.23	5.10	3.30	5.20	3.36	5.29	3.42	5.39	3.48	5.49	3.55	5.59	3.61	5.69	3.68
1.4	5.80	3.75	5.90	3.81	6.01	3.88	6.11	3.95	6.22	4.02	6.33	4.09	6.44	4.16	6.55	4.23	6.66	4.30	6.77	4.38
1.5	6.89	4.45	7.00	4.52	7.12	4.60	7.24	4.68	7.36	4.76	7.48	4.83	7.60	4.91	7.72	4.99	7.84	5.07	7.97	5.15
1.6	8.10	5.24	8.22	5.31	8.35	5.40	8.48	5.48	8.61	5.56	8.74	5.65	8.88	5.74	9.01	5.82	9.15	5.91	9.28	6.00
1.7	9.42	6.09	9.56	6.18	9.70	6.27	9.84	6.36	9.98	6.45	10.1	6.55	10.3	6.64	10.4	6.73	10.6	6.83	10.7	6.93
1.8	10.9	7.03	11.0	7.12	11.2	7.22	11.3	7.32	11.5	7.42	11.6	7.52	11.8	7.63	11.9	7.72	12.1	7.83	12.3	7.94
1.9	12.4	8.04	12.6	8.14	12.8	8.25	12.9	8.36	13.1	8.47	13.3	8.58	13.5	8.69	13.6	8.80	13.8	8.91	14.0	9.03
2.0	14.1	9.14	14.3	9.26	14.5	9.37	14.7	9.49	14.9	9.60	15.0	9.72	15.2	9.84	15.4	9.96	15.6	10.1	15.8	10.2

LEUPOLD & STEVENS, INC.
STEVENS WATER RESOURCES DATA BOOK. 2nd ED.

TABLE 2

FLOW THROUGH RECTANGULAR WEIRS WITH END CONTRACTIONS

Formula CFS = $3.33(L-0.2H)H^{3/2}$ MGD = CFS X .646317

Head	LENGTH OF WEIR CREST IN FEET											
	1		1½		2		3		4		5	
Ft.	CFS	MGD	CFS	MGD	CFS	MGD	CFS	MGD	CFS	MGD	CFS	MGD
.01	.003	.002	.005	.003	.007	.005	.010	.006	.013	.008	.017	.011
.02	.009	.006	.014	.009	.019	.012	.028	.018	.038	.025	.047	.030
.03	.017	.011	.026	.017	.035	.023	.052	.034	.069	.045	.086	.056
.04	.026	.017	.040	.026	.053	.034	.080	.052	.106	.069	.133	.086
.05	.037	.024	.055	.036	.074	.048	.111	.072	.149	.096	.186	.120
.06	.048	.031	.073	.047	.097	.063	.146	.094	.195	.126	.244	.158
.07	.061	.039	.092	.059	.122	.079	.184	.119	.246	.159	.307	.198
.08	.074	.048	.112	.072	.149	.096	.225	.145	.300	.194	.376	.243
.09	.088	.057	.133	.086	.178	.115	.268	.173	.358	.231	.448	.290
.10	.103	.067	.156	.101	.209	.135	.314	.203	.419	.271	.524	.339
.11	.119	.077	.180	.116	.240	.155	.362	.234	.483	.312	.605	.391
.12	.135	.087	.204	.132	.274	.177	.412	.266	.550	.355	.689	.445
.13	.152	.098	.230	.149	.308	.199	.464	.300	.620	.401	.776	.502
.14	.170	.110	.257	.166	.344	.222	.518	.335	.693	.448	.867	.560
.15	.188	.122	.284	.184	.381	.246	.575	.372	.768	.496	.961	.621
.16	.206	.133	.313	.202	.419	.271	.633	.409	.846	.547	1.059	.684
.17	.225	.145	.342	.221	.459	.297	.692	.447	.926	.598	1.159	.749
.18	.245	.158	.372	.240	.499	.323	.754	.487	1.008	.651	1.262	.816
.19	.265	.171	.404	.260	.541	.350	.817	.528	1.093	.706	1.368	.884
.20	.286	.185	.435	.281	.584	.377	.882	.570	1.179	.762	1.477	.955
.21	.307	.198	.468	.302	.627	.405	.948	.613	1.268	.820	1.589	1.027
.22	.329	.213	.501	.323	.672	.434	1.016	.657	1.359	.878	1.703	1.101
.23	.350	.226	.534	.345	.718	.464	1.085	.701	1.452	.938	1.820	1.176
.24	.373	.241	.568	.367	.764	.494	1.156	.747	1. 47	1.000	1.939	1.253
.25	.395	.255	.604	.390	.812	.525	1.228	.794	1.644	1.063	2.060	1.331
.26	.419	.271	.639	.413	.860	.556	1.301	.841	1.743	1.127	2.184	1.412
.27	.442	.286	.676	.437	.909	.588	1.376	.889	1.844	1.192	2.311	1.494
.28	.466	.301	.712	.460	.959	.620	1.453	.939	1.946	1.258	2.439	1.576
.29	.490	.317	.750	.485	1.010	.653	1.530	.989	2.050	1.325	2.570	1.661
.30	.514	.332	.788	.509	1.062	.686	1.609	1.040	2.156	1.393	2.703	1.747
.31	.539	.348	.827	.535	1.114	.720	1.689	1.092	2.263	1.463	2.838	1.834
.32	.564	.365	.866	.560	1.167	.754	1.770	1.144	2.373	1.534	2 975	1.923
.33	.590	.381	.905	.585	1.221	.789	1.852	1.197	2.483	1.605	3.115	2.013
.34	.615	397	.945	.611	1.275	.824	1.936	1.251	2.596	1.678	3.256	2.104
.35	.641	.414	.986	.637	1.331	.860	2.020	1.306	2.710	1.752	3.399	2.197

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TABLE 3

FLOW PER FOOT OF LENGTH THROUGH RECTANGULAR WEIRS WITHOUT END CONTRACTIONS

Formula CFS = $3.33 L H^{3/2}$ MGD = CFS x .646317

Head	.00		.01		.02		.03		.04		.05		.06		.07		.08		.09	
Ft.	CFS	MGD	CFS	MGD	CFS	MGD	CFS	MGD	CFS	MGD	CFS	MGD	CFS	MGD	CFS	MGD	CFS	MGD	CFS	MGD
0.0	.00	.00	.00	.00	.01	.01	.02	.01	.03	.02	.04	.03	.05	.03	.06	.04	.08	.05	.09	.06
.1	.11	.07	.12	.08	.14	.09	.16	.10	.17	.11	.19	.12	.21	.14	.23	.15	.25	.16	.28	.18
.2	.30	.19	.32	.21	.34	.22	.37	.24	.39	.25	.42	.27	.44	.28	.47	.30	.49	.32	.52	.34
.3	.55	.36	.57	.37	.60	.39	.63	.41	.66	.43	.69	.45	.72	.47	.75	.48	.78	.50	.81	.52
.4	.84	.54	.87	.56	.91	.59	.94	.61	.97	.63	1.01	.65	1.04	.67	1.07	.69	1.11	.72	1.14	.74
.5	1.18	.76	1.21	.78	1.25	.81	1.28	.83	1.32	.85	1.36	.88	1.40	.90	1.43	.92	1.47	.95	1.51	.98
.6	1.55	1.00	1.59	1.03	1.63	1.05	1.67	1.08	1.70	1.10	1.75	1.13	1.79	1.16	1.83	1.18	1.87	1.21	1.91	1.23
.7	1.95	1.26	1.99	1.29	2.03	1.31	2.08	1.34	2.12	1.37	2.16	1.40	2.21	1.43	2.25	1.45	2.29	1.48	2.34	1.51
.8	2.38	1.54	2.43	1.57	2.47	1.60	2.52	1.63	2.56	1.65	2.61	1.69	2.66	1.72	2.70	1.75	2.75	1.78	2.80	1.81
.9	2.84	1.84	2.89	1.87	2.94	1.90	2.99	1.93	3.03	1.96	3.08	1.99	3.13	2.02	3.18	2.06	3.23	2.09	3.28	2.12
1.0	3.33	2.15	3.38	2.18	3.43	2.22	3.48	2.25	3.53	2.28	3.58	2.31	3.63	2.35	3.69	2.38	3.74	2.42	3.79	2.45
1.1	3.84	2.48	3.89	2.51	3.95	2.55	4.00	2.59	4.05	2.62	4.11	2.66	4.16	2.69	4.21	2.72	4.27	2.76	4.32	2.79
1.2	4.38	2.83	4.43	2.86	4.49	2.90	4.54	2.94	4.60	2.97	4.65	3.01	4.71	3.04	4.77	3.08	4.82	3.12	4.88	3.15
1.3	4.94	3.19	4.99	3.23	5.05	3.26	5.11	3.30	5.17	3.34	5.22	3.37	5.28	3.41	5.34	3.45	5.40	3.49	5.46	3.53
1.4	5.52	3.57	5.58	3.61	5.63	3.64	5.69	3.68	5.75	3.72	5.81	3.76	5.87	3.79	5.93	3.84	6.00	3.88	6.06	3.92
1.5	6.12	3.96	6.18	3.99	6.24	4.03	6.30	4.07	6.36	4.11	6.43	4.16	6.49	4.19	6.55	4.23	6.61	4.27	6.68	4.32

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	TRAINING GUIDE NOTE	REFERENCES/RESOURCES

D.1

A float gage is shown in Fig. 3, installed in a stilling well for measurement of the head on the weir (H). The floor of the stilling well is level with the bottom of the channel in which the liquid is flowing. In order to use the gage to measure the head on the weir, it must be "zeroed" under a set of known conditions, which are, for purposes of illustration, assumed to be as shown in the figure. These conditions are as follows:

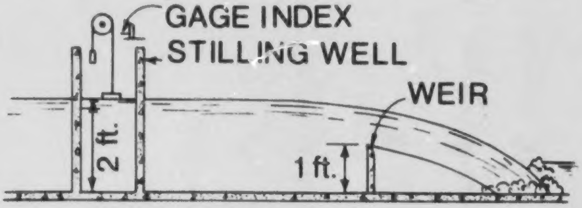


FIG. 3 - FLOAT GAGE INSTALLATION.

Height of the weir crest above the channel floor - 1 foot

Depth of liquid at the gage site - 2 feet

Tape reading opposite gage index - 3 feet

Under these conditions, it is known that the head on the weir equals one foot, i.e. liquid depth at the gage (2 feet) minus the distance from the floor of the channel to the weir crest (1 foot). Therefore 2 feet must be subtracted from the gage tape reading to obtain the head on the weir. Consequently, by subtracting 2 feet from the tape reading under any other condition, the head on the weir will be obtained.

The following points should be noted in connection with this procedure:

(a) If the elevation of the gage index is changed, the gage must be re-zeroed.

(b) If the position of the tape on the pulley is changed, the gage must be re-zeroed.

(c) The tape must be installed so that the numerical value of the tape reading increases as the depth of the flow increases.

A.3 A side view of a channel in which a weir has been installed is shown in Fig. 4.

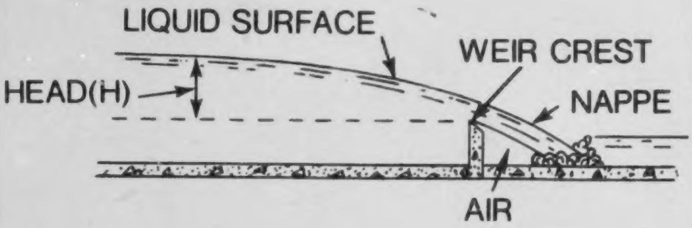


FIG. 4 - WEIR INSTALLATION

The following definitions apply:

Weir - A notch of regular form through which liquid flows.

Weir Crest - The edge over which the liquid flows.

Sharp-crested Weir - A weir with a sharp upstream edge so formed that the liquid springs clear of the crest

Head on Weir (H) - upstream depth of liquid over the crest of the weir. For a V-notch weir, the depth is measured from the bottom of the notch.

Nappe - the overflowing sheet of liquid.

Free Discharge (free-flow) - when nappe discharges into the air.

Submerged Discharge (submergence) - when liquid level downstream of the weir is at a higher elevation, or the same elevation as the weir crest, so that the nappe discharges partially under water.

Zero Gage Elevation - The division on the staff gage which is at the same level as the weir crest.

A.4 Weirs are designated according to the shape of the notch through which the liquid flows. The types of weirs most commonly used to measure wastewater flows are:

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.4 (Cont'd)	1. The 90° V-notch (triangular) weir, which has sides inclined 45° from the vertical.	

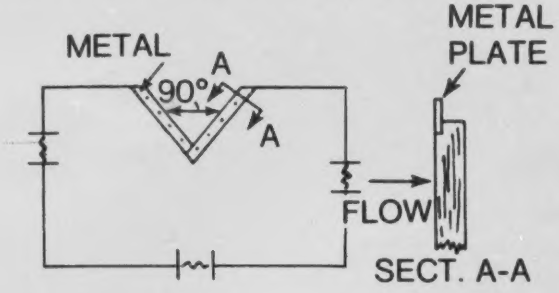


FIG. 5 - 90° V-NOTCH WEIR

(NOTE: Triangular weirs having notch angles other than 90° may also be used. Angles of 22-1/2°, 45°, and 60°, will sometimes be seen. Procedures for using these weirs are exactly the same as for the 90° weir, except that different formulas and weir tables apply. It is necessary that the proper formula or table be selected for the specific weir being used.)

2. The standard contracted rectangular weir, or weir with end contractions.

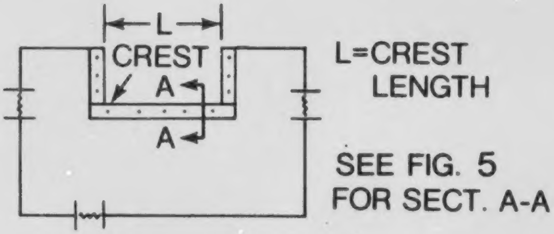
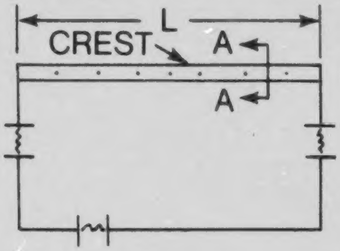


FIG. 6 - WEIR WITH END CONTRACTIONS

TRAINING GUIDE NOTE | REFERENCES/RESOURCES

A.4 (Cont'd) 3. The standard suppressed rectangular weir, or weir without end contractions.



L=CREST LENGTH

SEE FIG. 5 FOR SECT. A-A

FIG. 7 - SUPPRESSED WEIR

The weir notch or weir crest, as shown in the above illustrations, is cut with a sharp upstream edge into a relatively thin metal plate that is mounted on a supporting bulkhead. The crest should be 1-2 mm thick, (3/64 to 5/64 inch).

A.5 A standard staff gage, used for obtaining head measurements, is illustrated below:

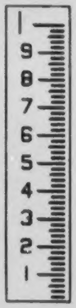


FIG. 8 - STAFF GAGE SECTION

Commercially-available gages are generally made of 18 gage metal coated with a substantial thickness of porcelain enamel. The standard gage is 4" wide and 3-1/3' long. The face of the gage is white; numerals and graduations are black. Gages may be made to any length desired, using similar details.

A.6 A float gage (Fig. 9) is a means of continuously indicating liquid levels. It consists of a metal float, a pulley mounted on a standard, and a

A.6 (Cont'd)

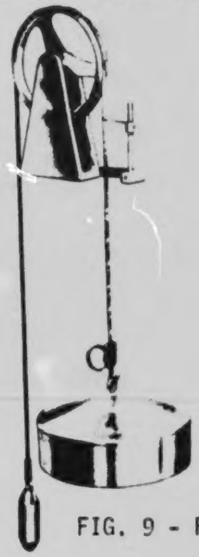


FIG. 9 - FLOAT GAGE

counterweight. A graduated stainless steel tape is attached to the float and connected at the other end to the counterweight. The float follows the rise and fall of the liquid surface and the level can be read from the tape and a pointer or reference mark. Tapes are available in selected lengths, and are graduated either in feet, tenths, and hundredths for English measurements, or meters, decimeters and centimeters for metric measurements.

The float gage is used extensively as a reference gage in stilling wells to check the accuracy of automatic head or flow recording devices.

B.1.1 The measured head will be too low if leakage of the liquid occurs along the sides or bottom of the bulkhead. All observed leaks should be immediately eliminated.

The upstream face of the bulkhead should be in a vertical plane perpendicular to the axis of the channel, for accurate results.

The bulkhead should be perpendicular to the direction of liquid flow, for accurate results.

B.1.2 The weir crest must be horizontal for standard formulas and weir tables to apply. The crest should be checked periodically, and leveled if required.

B.1.2 (Cont'd) The gage division which is at the elevation of the weir crest will be referred to as the "zero gage elevation." Its value must be known in order to calculate the head on the weir. When a weir installation is made, the zero gage elevation is determined, but, since this may change for some reason, it should be checked from time to time and a new zero elevation established, if necessary.

Small nicks and dents can reduce the accuracy of a weir installation. Those that do occur should be carefully dressed with a fine-cut file or stone, stroking only in the plane of the upstream weir face, the plane of the weir crest or sides, or the plane of the chamfers. Under no circumstances should the upstream corners of the notch be rounded or chamfered; nor should the shape of the weir opening be changed by attempting to completely remove an imperfection. Instead, only those portions of the metal that protrude above the normal surfaces should be removed. In extreme cases, replacement of the weir plate may be required.

Build up of extraneous material on the weir crest can cause inaccurate results. Such material should be cleaned off the weir plate prior to a head measurement.

B.1.3 If the liquid level downstream of the weir rises high enough so that there is no air space under the nappe, use of standard formulas and weir tables will produce inaccurate results. The nappe must be ventilated, i.e., have an air space underneath it. Do not attempt to use the weir as a measuring device if it is operating under a condition of submerged discharge.

If the nappe does not spring completely free of the weir, but clings to the downstream side wholly or in part, an inaccurate result will be obtained. The cause of such a condition must be determined, and the condition corrected, if good data are to be secured.

B.1.4 Any large submerged or floating objects in the channel upstream of the weir should be removed.

Sediment deposits behind the weir structure can affect the accuracy of the installation. Deposited material must be cleaned out when the vertical distance from the top of the deposit to the weir crest

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
	is one foot or less. More frequent cleaning is desirable.	
F.1.5	A disturbance of the normal flow pattern will affect the accuracy of a measurement made while such disturbance exists. If the normal flow is disturbed for any reason in connection with obtaining a head reading, adequate time should be allowed before making the reading, so that normal conditions may be re-established.	

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

AMPEROMETRIC DETERMINATION OF FREE AND COMBINED RESIDUAL CHLORINE IN WATER

as applied in

WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

Developed by the

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

EFFLUENT MONITORING PROCEDURE: Amperometric Determination of Free and Combined Residual Chlorine in Water

This instructional sequence was developed by:

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EDUCATION AND TECHNICAL BACKGROUND

B.S. - Chemistry

14 years Industrial Chemist

16 years HEW-FWPCA-EPA-Chemist

EFFLUENT MONITORING PROCEDURE: Amperometric Determination of Free and Combined Residual Chlorine in Water

1. Analysis Objectives

The operator will be able to perform an amperometric titration for the determination of free and combined residual chlorine in water.

2. Brief Description of Analysis*

Free available residual chlorine and combined residual chlorine are titrated successively using an amperometric titrator. The free available residual chlorine is titrated first. The sample pH is then dropped to 4 by adding buffer solution pH 4 and then potassium iodide is added to the sample. The first titration will represent the free available residual chlorine while the second titration will represent the combined residual chlorine.

3. Applicability of this Procedure:

a. Range of Concentration:

Chlorine residuals over 2 mg/l are best measured by means of smaller samples, or by dilution with water that neither is chlorinated nor has a chlorine demand.

b. Pretreatment of Samples:

None

c. Treatment of Interferences in Samples:

None

*Standard Methods for the Examination of Water and Wastewater, 14th Ed., 1975. APHA, Washington, D.C., p. 322

EFFLUENT MONITORING PROCEDURE: Amperometric Determination of Free and Combined Residual Chlorine in Water

General Description of Equipment used in the Process

A. Capital Equipment

1. Amperometric Titrator Assembly - Wallace and Tiernan*

B. Reusable

1. 1 pipette (1 ml capacity)
2. 1 pipette (5 ml capacity)
3. 1 sample cup (to contain 200 ml)
4. 1 plastic squeeze bottle

C. Consumable**

1. 1 bottle phenylarsene oxide solution 0.00564 N (16 ounce)
2. 1 bottle pH 4 buffer solution (4 ounce)
3. 1 bottle pH 7 buffer solution (4 ounce)
4. 1 bottle potassium iodide solution (4 ounce)
5. 1 bottle sodium chloride electrolyte tablets (8 ounce)

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*Mention of a specific brand name does not constitute endorsement by the U.S. Environmental Protection Agency

**Consumable reagents listed are available from Wallace & Tiernan Industrial Products Division, 25 Main St., Belleville, NJ 07109

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Equipment Preparation	1. Set up titrator on work bench.	1a. Electric outlet 110 volt required. 1b. Amperometric titrator assembly available from Wallace and Tiernan Corporation	
	2. Select proper pipette for titration.	2a. Two pipettes are furnished with the titrator. The 1 ml pipette is generally used when the residual is less than 1 mg/l. A 5 ml pipette is for use with higher residuals.	
	3. Lightly grease the lower end and insert it in the top of the pump unit on the side of the titrator.	3a. Use silicone grease or other similar lubricant.	
	4. Fill the pump squeeze bottle about 2/3 to 3/4 full with phenylarsene oxide solution.	4a. Reagent is highly toxic - avoid ingestion.	
	5. Screw the bottle on to the pump.	5a. It is easier to turn the bottle than the cap.	
	6. Pour sufficient electrolyte tablets into the cell unit to fill the chamber about 2/3 full.		VII.A.6 (p. 15)
	7. Add enough distilled water to cover the tablets.	7a. Use a plastic squeeze bottle.	
	8. Plug the cell unit into the titrator.	8a. The cell is so designed that it cannot be plugged in except in the correct position.	V.A.8.8a (p. 13)
	9. Examine the titrator cup. The cup has a line indicating the 200 ml level.	9a. Whenever the term "sample" is used in these instructions it shall mean a 200 ml volume of the water to be tested.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Determination of Free Available Residual Chlorine	1. Plug the electric power plug into a source of 115 volt, single phase, 60 cycle A.C. current.		I.B.1 (p. 13)
	2. Fill the pipette with phenylarsene oxide solution.	2a. Alternately squeeze and release the squeeze bottle.	
	3. Remove all air from the pipette and plastic tubing by rotating the red knob in the stem unit 1/4 turn counter-clockwise.	3a. The pipette should drain through the plastic tubing.	
	4. Catch the discarded solution in a 50 ml beaker.		
	5. Refill the pipette to the top (zero) calibration mark.		
	6. Add sample water to the cup. Adjust the level to the line.	6a. The volume of sample is 200 ml.	
	7. Place the cup on the titrator.	7a. The top edge of the cup should go behind the cup guide post. 7b. The bottom of the cup should rest on the support post. 7c. The plastic tubing from the pump should be submerged in the sample about 1/16 inch. If necessary, adjust the tubing on the guide post to obtain this condition.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
	8. Add 1 ml of buffer solution pH 7 to the water sample.	8a. The droppers furnished with the titrator are 1 ml units. A dropper full of solution should be used wherever 1 ml of solution is called for. 8b. If the pH of the sample is between 6.0 and 7.5 it is not necessary to add buffer.	
	9. Start the agitator by turning the switch to "ON".		
	10. Adjust the meter to make the pointer read maximum on the scale.	10a. Rotating the adjusting knob clockwise should increase the reading. 10b. If the pointer is above maximum when the adjusting knob is rotated completely counter-clockwise, then the titration should be started with the knob in this position.	
	11. Start adding small amounts of titrant and note the deflection of the meter scale after each addition.	11a. If free available chlorine is present in the sample and if the pointer is on scale at the beginning of the titration, then the first addition of titrant should cause a definite pointer movement to the left. If the pointer goes below zero then it should be brought back on scale by rotating the adjusting knob clockwise.	
	12. Continue the addition of small amounts of titrant until the addition of titrant no longer causes a deflection of the needle.	12a. In most waters the end-point of the reaction is just passed when the addition of a small amount of titrant no longer deflects the pointer to the left. 12b. The amount of titrant used in the titration is then read from the pipette and the last increment is subtracted from the pipette reading and the resultant figure represents the free available residual chlorine in mg/l.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
	13. Subtract the last reading from the previous reading.		
	14. The reading on the pipette represents the amount of free available chlorine in mg/l.		
	15. Turn instrument "OFF".		
	16. Record your result.		
C. Determination of combined residual chlorine	1. Repeat steps 1 through 7 of the free available chlorine procedure if the free available chlorine determination has not been performed.	1a. The general procedure for measuring total residual chlorine is the same as that given for measuring free available residual chlorine.	
	2. If you have just completed the free chlorine determination, you can continue the use of the same sample for this determination.		
	3. Add 1 ml of buffer solution pH 4 to the sample.	3a. Use the dropper to add the buffer solution.	
	4. Add 1 ml of potassium iodide solution to the water sample.	4a. Use the dropper to add the potassium iodide solution.	
		4b. When potassium iodide is added, the pointer may first deflect to the left and then go up-scale.	VII.C.4.4b (p. 14)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
	5. Follow steps 9 through 16 of the previous procedure for the determination of free available chlorine. In this case the result is reported as combined residual chlorine.	5a. Free available residual chlorine and combined residual chlorine may be measured in one sample by combining the two procedures. 5b. The free available chlorine is measured first. The sample pH is then dropped to 4 by adding buffer solution pH 4 and then potassium iodide. 5c. If combined residual chlorine is present, the pointer will deflect to the right when potassium iodide is added. 5d. The first titration will represent the free available residual chlorine while the second titration will represent the combined residual chlorine.	

EFFLUENT MONITORING PROCEDURE: Amperometric Determination of Free and Combined Residual Chlorine in Water

TRAINING GUIDE

SECTION	TOPIC
I*	Introduction
II	Educational Concepts - Mathematics
III	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field and Laboratory Equipment
VI	Field and Laboratory Reagents
VII*	Field and Laboratory Analysis
VIII	Safety
IX	Records and Reports

*Training guide materials are presented here under the heading marked *. These standardized headings are used throughout this series of procedures.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
I.B.1	The fundamental chemical procedure involved in the amperometric titrator is the neutralization of an oxidizing agent (free available chlorine) in a sample of water by the addition of a reducing agent of known strength. Immersed in the sample cell unit which produces a small direct current which is proportional to the free chlorine present in the sample. The current is indicated on a microammeter which is connected to the cell unit. As the reducing agent is added, the amount of free chlorine is reduced, the cell current decreases, and the microammeter pointer moves down scale. The end point of the reaction occurs when enough reducing agent has been added to just neutralize all of the free chlorine in the sample. When this point is reached, the further addition of a small amount of reducing agent no longer deflects the pointer to the left. On the titrator, the sample volume and the strength of the reducing agent have been selected to make 1 milliliter of reducing agent equivalent to one milligram per liter of chlorine. When the endpoint is reached, therefore, the volume of reducing agent used represents the chlorine concentration in mg/l.	
	Under the conditions specified in the titration procedure, the titration can be used to distinguish between free available residual chlorine and combined residual chlorine because the reducing agent employed reacts readily with free chlorine but does not react with combined chlorine. If either combined or total residual chlorine is to be measured, potassium iodide is added to the sample to produce an amount of free iodine which is equivalent to the original residual chlorine. The reducing agent reacts readily with free iodine so that the titration can be carried out in a manner similar to that used for free available residual chlorine determination.	
V.A.8.8a	The electrolyte used in the inner chamber of the cell has a tendency to crystallize out on the contact springs and in the terminals of the cell unit. This may slightly corrode	

the electrical contacts between the various units. Improper electrical connections cause erratic microammeter pointer readings during the titration. Should any crystals accumulate on the plastic cell unit, these parts should be washed off with warm water. CAUTION: Never use water warmer than 100°F, as hot water softens the plastic. When the titrator is not to be used for extended periods, the cell unit should be washed out to remove all electrolyte tablets and solution, and stored dry.

VII.C.4.4b If free available residual chlorine determinations are to be made after potassium iodide has been used in preceding titrations, the cell unit should be rinsed off in several sample cups of water to remove traces of potassium iodide solution and buffer solution pH 4.

Occasionally, when potassium iodide is added to the sample, the pointer will drop to the left and will not come back on scale even though the poteniometer is turned completely clockwise. Under these conditions, the cell unit is said to have lost its sensitivity to iodine. This situation is likely to arise if the titrator has been used to determine free chlorine only for extended periods of time, i.e., the cell unit has not been exposed to iodine for prolonged periods.

The sensitivity of the cell unit can be restored by adding enough free iodine to the distilled water in the sample jar to create a yellowish color. The free iodine may be in the form of tincture of iodine or may be obtained by adding potassium iodide to a strong chlorine solution. Agitate the sample for two or three minutes and then allow the cell unit to stand in the iodine solution for 10 to 15 minutes. After this treatment, the cell unit should be rinsed off throughly to remove all traces of iodine.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
VII.A.6	The main requirement as far as electrolyte tablets are concerned is to have saturated electrolyte solution inside the cell unit at all times. Theoretically, this requirement is not as long as any tablets and water are in the cell unit. The actual water level inside the cell unit cannot be controlled since this level tends to equalize with (or even go below) the water level in the sample jar through the porous wicking.	

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

AMPEROMETRIC DETERMINATION OF TOTAL RESIDUAL CHLORINE IN WASTEWATER

as applied in

WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

Developed by the

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

EFFLUENT MONITORING PROCEDURE: Amperometric Determination of Total Residual Chlorine in Wastewater

This instructional sequence was developed by:

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POSITION	Chemist-Instructor

EDUCATION AND TECHNICAL BACKGROUND

B.S. - Chemistry

14 years Industrial Chemist

16 years HEW-FWPCA-EPA-Chemist

EFFLUENT MONITORING PROCEDURE: Amperometric Determination of Total Residual Chlorine in Wastewater

1. Analysis Objectives

The operator will be able to perform an amperometric titration for the determination of total residual chlorine in a sample of wastewater treatment plant effluent.

2. Brief Description of Analysis*

Residual chlorine present in wastewater is in the form of combined chlorine. A "Back-Titration" procedure is used to determine the phenylarsene oxide excess and a formula used to calculate the concentration of total residual chlorine in the sample.

3. Applicability of this Procedure:

a. Range of concentrations:

Applicable to all types of wastewater.

b. Pretreatment of Sample

None

c. Treatment of Interference in Samples.

Manganic Manganese, in concentrations as low as 1.0 mg/liter liberates iodine from iodide at a pH of 4.0.

Chromates reduce phenylarsine oxide. Method not applicable when high concentrations of chromates are present.

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*Annual Book of ASTM Standards, 1975. American Society for Testing and Materials. 1916 Race St., Philadelphia, PA 19103. p. 278.

EFFLUENT MONITORING PROCEDURE: Amperometric Determination of Total Residual Chlorine in Wastewater

General Description of Equipment used in the Process

A. Capital Equipment

1. Amperometric Titrator Assembly - Wallace and Tiernan*

B. Reusable

1. 1 pipette (1 ml capacity)
2. 1 pipette (5 ml capacity)
3. 1 sample cup (to contain 200 ml)
4. 1 plastic squeeze bottle

C. Consumable**

1. 1 bottle phenylarsene oxide solution 0.00564N (16 ounce)
2. 1 bottle pH 4 buffer solution (4 ounce)
3. 1 bottle pH 7 buffer solution (4 ounce)
4. 1 bottle potassium iodide solution (4 ounce)
5. 1 bottle sodium chloride electrolyte tablets (8 ounce)
6. Standard iodine solution 0.1 N
7. Standard iodine titrant 0.0282 N
8. Potassium iodide crystals
9. Iodine crystals, purified

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*Mention of a specific brand name does not constitute endorsement by the U.S. Environmental Protection Agency

**Consumable reagents listed are available from Wallace & Tiernan Industrial Products Division, 25 Main St., Belleville, NJ 07109

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Reagents Standard iodine solution 0.1 N	1. Dissolve 40.0 grams of potassium iodide (KI) in 50 ml of distilled water. Add 12.7 g. of iodine crystals and stir until solution is complete.		
	2. Dilute to one liter with distilled water.	2a. Store the solution in a dark bottle.	
Iodine standard solution (0.0282N)	3. Transfer 25 grams of potassium iodide into a one liter volumetric flask.	3a. Use a trip balance.	
	4. Add 200 ml of distilled water and swirl to dissolve.	4a. Use a graduate cylinder.	
	5. Add 285 ml of 0.1 N iodine solution and dilute to the mark with distilled water.		
B. Determination of total residual chlorine	1. Set up titrator and plug into a source of 115 volt, single phase, 60 cycle A.C. current.		
	2. Add sample water to the cup. Adjust the level to the line.	2a. The volume of sample is 200 ml.	
	3. Place the cup on the titrator.	3a. The top edge of the cup should go behind the cup guide post. 3b. The bottom of the cup should rest on the support post.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Continued	4. Turn the switch to start the agitator.		
	5. Add 5 ml of phenylarsene oxide solution to the sample and mix.	5a. Use a 5 ml pipette.	
	6. Add 4.0 ml of pH 4.0 buffer solution to the sample and mix.	6a. Should be sufficient to insure a sample pH between 3.5 and 4.2.	
	7. Add 1.0 ml of KI solution.		
	8. Rotate the adjusting knob so that the microammeter pointer reads about 20 on the scale.		
	9. Add 0.0282 N iodine solution in small increments.	9a. Use a 1 ml volumetric pipette graduated in 0.1 ml. 9b. The standard reagent bottle, pump, pipette, and applicator tubing cannot be used for this purpose since the plastic components may react with the iodine solution and change its strength. 9c. As iodine is added to the sample, the pointer remains practically stationary until the end-point is approached. Just before the true end-point each increment of iodine solution causes a temporary deflection of the microammeter to the right, but the pointer drops back to about its original position. The true end-point is reached when a small addition of iodine solution gives a definite and permanent pointer deflection to the right (up-scale).	
	0. Note the volume of iodine solution used to reach the end-point.	0a. Calculate the total residual chlorine as follows: $\text{mg/l chlorine} = \left[\begin{array}{l}\text{total}\\ \text{phenylarsene}\\ \text{oxide used}\\ \text{(step 5)}\end{array}\right] - \left[\begin{array}{l}\text{(5)(ml of iodine)}\\ \text{used in}\\ \text{titration}\end{array}\right]$	

410

411

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Continued		10b. Example of calculation: 1. Total phenylarsene oxide used in step 5 = 5.0 ml. 2. ml of iodine required to reach the end-point in step 9 = 0.6 ml $\text{mg/l chlorine} = 5.0 - (5)(0.6)$ $= 5.0 - 3.0$ $= 2.0$	
		10c. The accuracy of the above procedure depends on the volume of the sample (step 2), the strength of the phenylarsene oxide solution (0.00564N) which is quite stable, and the strength of the iodine solution (0.0282 N) which is subject to deterioration with time. If the iodine is not 0.0282 N, it must be standardized by the following procedure.	
C. Standardization of iodine solution	1. Add 5.0 ml of phenylarsene oxide solution to 195 ml of dechlorinated water.	1a. Chlorine-demand-free water: add sufficient chlorine to distilled water to destroy the ammonia. The amount of chlorine required will be about ten times the amount of ammonia nitrogen present; in no case produce an initial residual of less than 1.0 mg/l free chlorine. Allow the chlorinated water to stand overnight or longer; then expose to direct sunlight until all residual chlorine is discharged. Use distilled water free from ammonia and nitrite to produce the chlorine demand-free water. Check chlorine residual by amperometric titration.	
	2. Titrate with the iodine solution.	2a. The end point is reached when a small addition of iodine gives a pointer deflection to the right (up scale) which holds for 15 to 20 seconds. If 1.0 ml of iodine solution neutralizes the 5.0 ml of phenylarsene oxide solution, the iodine solution	

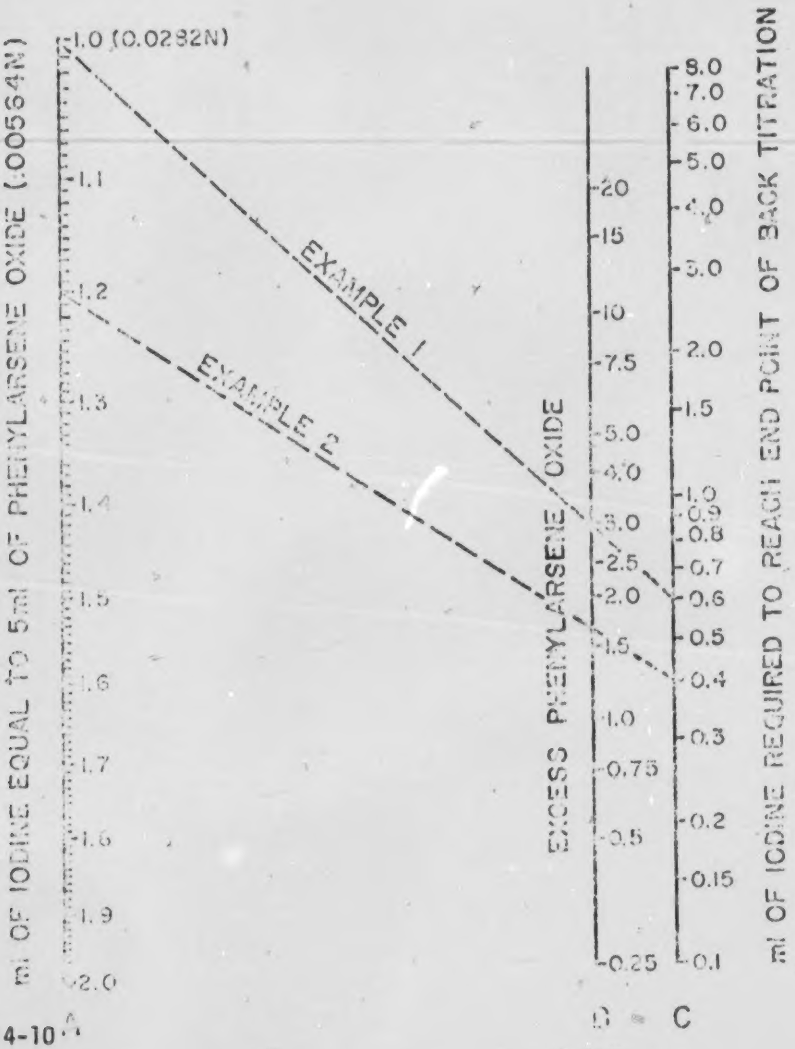
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
		11a. Continued is 0.0282 N. If the iodine solution has deteriorated, the volume of iodine solution to reach the end-point (something greater than 1.0 ml) is equal to 5 ml of phenylarsene oxide solution. 11b. "Back titration" for residual chlorine may be made with weaker than 0.0282 N iodine solutions. The attached chart can be used to determine the excess phenylarsene oxide by following step 12 and subsequent steps.	
	12. Locate on line "A" the ml of iodine equal to 5.0 ml of phenylarsene oxide.		
	13. Locate on line "C" the volume of iodine as determined in step 9.		
	14. Determine where a line connecting these points crosses line "B".	14a. This is the excess phenylarsene oxide. 14b. As expressed in the formula, the mg/l of chlorine residual is the excess phenylarsene oxide subtracted from the total. 14c. Example of calculation: 1. Total phenylarsene oxide = 10.0 ml 2. ml iodine equal to 5.0 ml phenylarsene oxide = 1.2 ml. 3. ml iodine to reach end point of "back titration" = 0.4 4. Excess phenylarsene oxide (from chart) = 1.6 (approx.) 5. mg/l chlorine residual = 10-1.6 = 8.4	

EXAMPLE

Total phenylarsene oxide =5 ml.
ml. iodine equal to 5 ml. phenylarsene oxide =1 ml. (0.0282N solution)
ml. iodine to reach end point of "Back Titration" = 0.6
Excess phenylarsene oxide (from chart) = 3.0
or (from formula) = 5 x 0.6 =3.0
ppm chlorine residual = 5 - 3 =2

EXAMPLE

Total phenylarsene oxide =10 ml.
ml. iodine equal to 5 ml. phenylarsene oxide =1.2 ml.
ml. iodine to reach end point of "Back Titration" =0.4
Excess phenylarsene oxide (from chart) =1.6 (approximately)
ppm chlorine residual =10 - 1.6=8.4



A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

TITRIMETRIC DETERMINATION OF TOTAL RESIDUAL CHLORINE IN WASTEWATER EFFLUENTS

as applied in

WASTEWATER TREATMENT FACILITIES
AND IN THE
MONITORING OF EFFLUENT WASTEWATERS

Developed by the

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

EFFLUENT MONITORING PROCEDURE: Titrimetric Determination of Total Residual Chlorine in Wastewater

This operational procedure was developed by:

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POSITION Chemist-Instructor

EDUCATION AND TECHNICAL BACKGROUND

B.S. - Chemistry

M.S. - Chemistry

1-1/2 years Industrial Chemist

4 years additional Graduate School

4 years college Chemistry Instructor

1-1/2 years DHEW - Air Pollution Program, Chemist

10 years DI - EPA, Chemist-Instructor

EFFLUENT MONITORING PROCEDURE: Titrimetric Determination of Total Residual Chlorine in Wastewater Effluents

1. Analysis Objectives:

The learner will determine the total residual chlorine in a sample of wastewater treatment plant effluent.

2. Brief Description of Analysis:

Chlorine, hypochlorus acid, and hypochlorite ion are collectively referred to as free chlorine. Free chlorine is added to wastewater effluents for disinfection purposes. It combines with ammonia in the effluent to form monochloramine, dichloramine, and nitrogen trichloride; these three compounds together, are called combined chlorine. The sum of combined and free chlorine is referred to as total residual chlorine. In this procedure, called a back-titration, an amount of reducing agent (phenylarsene oxide) more than sufficient to react with the total residual chlorine is added to the sample. The amount of excess reducing agent is then determined by titration with standard iodine solution. The result is expressed as mg of total residual chlorine per liter of sample.

3. Applicability of this Procedure:

a. Range of Concentration:

Although the cited reference* does not specifically mention the range of applicability, it can be inferred from the procedure that concentrations of up to 10 mg total residual chlorine/liter can be accurately analyzed. No inference can be made about the lower limit of the test.

b. Sample Pretreatment:

None. The determination must be carried out immediately after sampling. Avoid exposure of the sample to strong sunlight and excessive agitation.

c. Treatment of Interferences:

The interference due to manganese, iron, and nitrite is minimized by buffering the reaction mixture as described in this procedure. If the sample contains a large amount of organic matter, the titration endpoint may be obscured. This problem may be overcome by acidifying the reaction mixture to a pH of 1.0, but only if the sample contains no manganese, iron, or nitrite. If they are present, then the amperometric procedure should be used.

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* Source of Procedure: Standard Methods, 14th ed., method 409 B., page 318.

EFFLUENT MONITORING PROCEDURE: Titrimetric Determination of Total Residual Chlorine in Wastewater Effluents

General Description of Equipment Used in the Process

A. Capital Equipment:

1. Trip balance, 100 g capacity
2. Analytical balance
3. Still, or other source of distilled water

B. Reusable Supplies:

1. Brushes (for cleaning glassware)
2. Brush (for cleaning balance)
3. Laboratory apron
4. Safety glasses
5. One distilled water plastic squeeze bottle
6. One pen or pencil
7. One notebook (for recording data)
8. Sponge (for cleaning laboratory table top)
9. One 2 liter Erlenmeyer flask
10. One 500 ml Erlenmeyer flask
11. One 250 ml Erlenmeyer flask
12. One 125 ml Erlenmeyer flask
13. One 1 liter graduated cylinder
14. One 500 ml graduated cylinder
15. One 250 ml graduated cylinder
16. One 100 ml graduated cylinder
17. One 10 ml graduated cylinder
18. Six 1 liter glass-stoppered bottles
19. One 1 liter plastic bottle
20. Two 100 ml glass-stoppered bottles
21. One 1 liter volumetric flask
22. One 50 ml volumetric pipet
23. One 10 ml volumetric pipet
24. One 5 ml volumetric pipet
25. One 10 ml graduated pipet
26. One 250 ml beaker
27. One 30 ml beaker
28. One 2 liter beaker (for cleaning glassware)
29. One small spatula (for use when weighing solids)
30. One hot plate (to accommodate a 2 liter Erlenmeyer flask)
31. One mortar and pestle (about 100 ml capacity)
32. One 50 ml buret
33. One 5 ml buret
34. One small powder funnel (to fit into the top of a 1 liter volumetric flask)
35. One small funnel (to fit in the top of the buret)
36. One clamp (to support the buret)
37. One ring stand (for use with the buret and clamp)
38. Magnetic stirrer and 2 inch stirring bar (optional)
39. One weighing bottle with top (about 15 ml capacity)
40. Fifteen inches of 6 mm glass tubing
41. Two feet of tygon tubing (to connect the lecture bottle of carbon dioxide to the 6 mm glass tubing)

42. One universal clamp (to support the lecture bottle on the ring stand)
43. One eyedropper
44. One grease pencil
45. One pH meter (with pH 4 & 7 buffers)
46. Six inch stirring rod
47. Sufficient aluminum foil to wrap a 1 liter glass-stoppered bottle
48. One asbestos glove or towel (to facilitate lifting a flask of hot water)

C. Consumable Supplies:

1. Concentrated sulfuric acid, H_2SO_4
2. Sodium dichromate, $Na_2Cr_2O_7$
3. Soap
4. Eight plastic weighing boats (about 2 inches square)
5. 76 g of potassium iodide, KI
6. 5 g of soluble starch
7. 1.25 g salicylic acid, 2-$HOC_6H_4CO_2H$
8. 5 g of arsenic trioxide, As_2O_3
9. 27 g of sodium hydroxide, NaOH
10. Lecture bottle of carbon dioxide, CO_2
11. 13 g of resublimed (some catalogs simply use the word sublimed) iodine, I_2
12. 55 ml of concentrated (12N) hydrochloric acid, HCl
13. 0.8 g of phenylarsine oxide powder, C_6H_5AsO
14. 146 g of anhydrous sodium acetate, $NaC_2H_3O_2$ or 243 g of sodium acetate trihydrate, $NaC_2H_3O_2 \cdot 3H_2O$

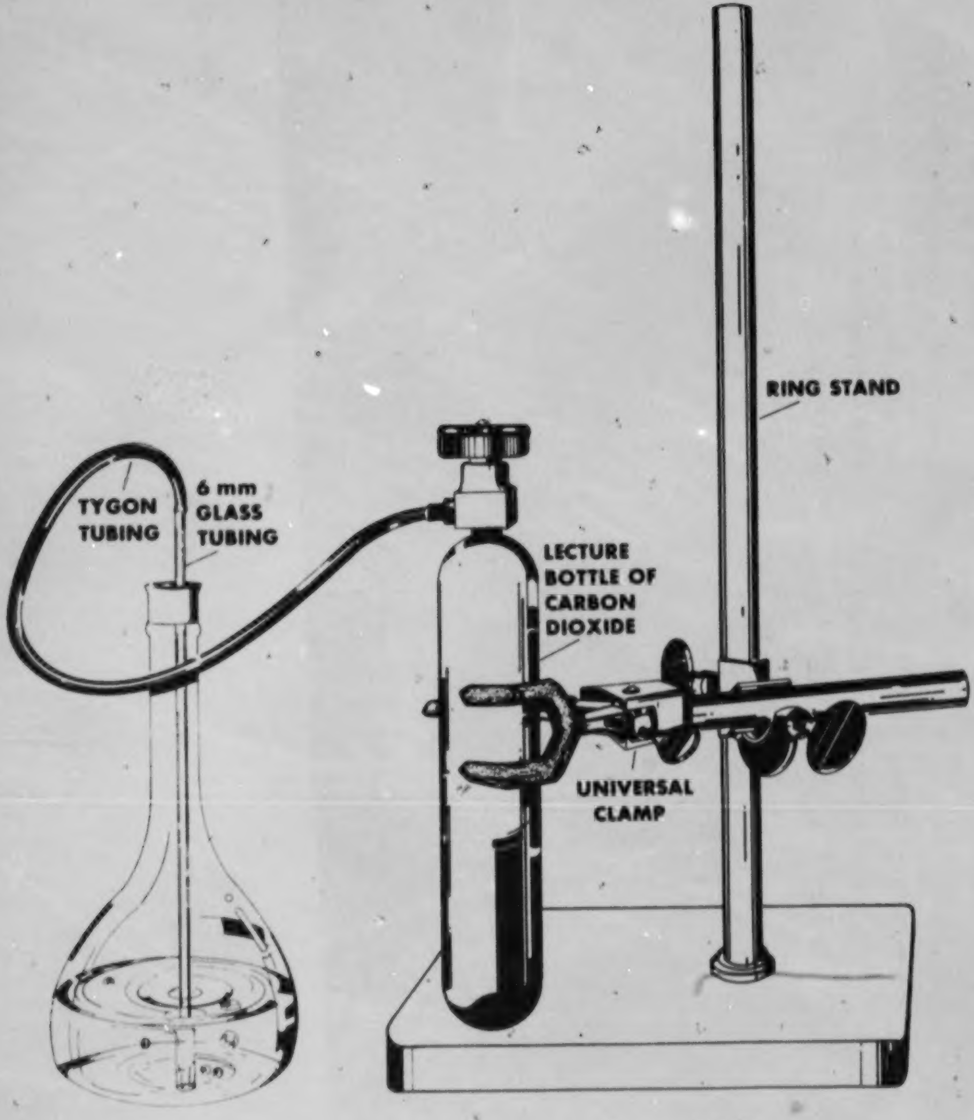
Items C.1., C.2., and C.3. are for cleaning glassware. The quantities needed will therefore vary.

Items C.5. through C.14 (except C.10.) are the exact amounts needed. To facilitate weighing solids and measuring liquids, include slightly more than the required amounts.

EFFLUENT MONITORING PROCEDURE: Titrimetric Determination of Total Residual Chlorine in Wastewater Effluents

All reagents should be of high quality. Different chemical manufacturers may have different ways of indicating a high quality reagent. While no endorsement of one chemical manufacturer over another is intended, the following are some designations used in four chemical catalogs to indicate high quality reagents.

Catalog	Designations
Thomas	Reagent, ACS, Chemically Pure (CP)
Matheson, Coleman & Bell	Reagent, ACS
Curtin Matheson Scientific, Inc.	Primary Standard, ACS, AR
Fisher	Certified, ACS


RING STAND
TYGON TUBING
6 mm GLASS TUBING
LECTURE BOTTLE OF CARBON DIOXIDE
UNIVERSAL CLAMP

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Equipment Preparation			
1. Cleaning of glassware	1. Clean all glassware and rinse with distilled water.	1a. Throughout this procedure, unless otherwise stated, the term water means distilled water.	V.A.1.1
2. Balance inspection	1. Check the analytical and trip balances for cleanliness and proper operation.	1a. Consult the manufacturer's manual for assistance in correcting any malfunction.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation			
1. Starch Indicator	1. Weigh 5 g of soluble starch.	1a. Use a trip balance.	
	2. Transfer it to a mortar.		
	3. Measure 1 liter of water.	3a. Use a 1 liter graduated cylinder.	
	4. Pour the water into a 2 liter Erlenmeyer flask.		
	5. Bring the water to a boil.	5a. Use a hot plate. 5b. While the water comes to a boil, do steps 6 & 7.	
	6. Add 1 ml of water to the starch in the mortar.	6a. Use a 10 ml graduated cylinder to measure the water.	
	7. Grind the starch and water together.	7a. Use a pestle. 7b. The objective is to form a thin paste. 7c. A few additional drops of water may have to be added.	
	8. Slowly pour the thin paste into the boiling water.	8a. Be cautious about the hot flask. 8b. If the water has not yet come to a boil, wait until it does.	
	9. Invert a 250 ml beaker and place it on top of the Erlenmeyer flask.	9a. As protection against contamination.	
	10. Turn the hot plate off.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)	11. Remove the flask from the hot plate.	11a. Caution: the flask is hot.	
	12. Allow the starch to stand overnight.	12a. Prepare the other reagents while the starch solution is standing.	
	13. Carefully decant the supernatant liquid into a 1 liter glass-stoppered bottle.		
	14. Weigh 1.25 g of salicylic acid.	14a. Use an analytical balance (or trip balance if it weighs to the second decimal place).	
	15. Add it to the bottle.		
	16. Swirl the bottle.	16a. To dissolve the salicylic acid.	
2. Potassium Iodide, KI, 10%	1. Weigh 10 g of potassium iodide, KI	1a. Fourteenth Standard Methods does not specify the strength of this solution. Ten % is the author's opinion.	
	2. Transfer it to a 250 ml Erlenmeyer flask.		
	3. Measure 90 ml of water.	3a. Use a 100 ml graduated cylinder.	
	4. Add it to the flask.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)	5. Swirl the flask.	5a. To dissolve the potassium iodide.	
	6. Transfer the solution to a 100 ml glass-stoppered bottle.		
3. Standard Arsenite, 0.1N	1. Wipe a weighing bottle with a tissue.	1a. To remove fingerprints. 1b. Throughout B.3., always handle the bottle with a tissue so as to avoid fingerprints.	
	2. Weigh about 5 g of arsenic trioxide, As_2O_3, in the weighing bottle.	2a. To four places to the right of the decimal. 2b. Arsenic trioxide, As_2O_3, is extremely toxic. Wipe up any spilled powder with a damp tissue, discard the tissue, and thoroughly wash your hands with soap and water.	
	3. Remove the bottle from the balance.		
	4. Fill a 1 liter volumetric flask about one-half full of water.		
	5. Place a small powder funnel into the mouth of the flask.		
	6. Remove the top of the weighing bottle.		
	7. Carefully turn the bottle upside down into the funnel in the volumetric flask.	7a. The arsenic trioxide is powdery, and will tend to "fly around"; so do this step carefully.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)			
	8. Gently tap the bottom and sides of the weighing bottle.	8a. So as to knock more of the arsenic trioxide into the funnel. 8b. Some of the solid will stay in the bottle.	
	9. Remove the weighing bottle from the funnel.	9a. Remember the arsenic trioxide toxicity when you later wash the bottle.	
	10. Replace the top of the weighing bottle.		
	11. Reweigh the weighing bottle.	11a. Use the same analytical balance that you used before. 11b. Record the weight to four places to the right of the decimal point.	
	12. Using a plastic squeeze bottle of water, carefully wash the arsenic trioxide down into the volumetric flask and remove the funnel.	12a. Use a minimum of water.	
	13. Measure 100 ml of water.	13a. Use a 100 ml graduated cylinder.	
	14. Weigh 15 g of sodium hydroxide, NaOH.	14a. Use a trip balance.	
	15. Add the sodium hydroxide and 100 ml of water to the volumetric flask.		
	16. Swirl the flask gently.	16a. To dissolve the sodium hydroxide and arsenic trioxide.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)			
	17. Add 150 ml of water to the flask.	17a. Use a 100 ml graduated cylinder.	
	18. Gently swirl the flask.	18a. To thoroughly mix the contents.	
	19. Insert the glass tube leading from the lecture bottle of carbon dioxide, CO_2, into the volumetric flask; see the figure on page	19a. The glass tube should extend to the bottom of the flask.	
	20. Carefully open the lecture bottle valve and adjust the flow of carbon dioxide so that about 1 bubble per second comes from the end of the tube.		
	21. Continue the addition of carbon dioxide for about 15 minutes.		
	22. Remove the tube from the flask.		
	23. Close the lecture bottle valve.		
	24. Add water to the 1 liter mark of the flask.		
	25. Thoroughly mix the contents of the flask.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)			
	26. Transfer the solution to a 1 liter glass-stoppered bottle.	26a. The arsenic trioxide solution is also extremely toxic. If any is spilled on the skin, rinse it off immediately with large amounts of tap water.	
	27. Calculate the strength (normality, N) of the arsenic trioxide.	27a. $N = \frac{A - B}{49.455}$ N = the strength (normality, N) of the arsenic trioxide. A = the weight, in g, of the weighing bottle + top + arsenic trioxide. B = the weight, in g, of the weighing bottle + top + arsenic trioxide residue.	
		27b. The arsenic trioxide solution is stable almost indefinitely.	
4. Standard Iodine 0.1N	1. Weigh 40 g of potassium iodide, KI.	1a. Use a trip balance.	
	2. Transfer it to a 250 ml Erlenmeyer flask.		
	3. Measure 25 ml of water.	3a. Use a 100 ml graduated cylinder.	
	4. Add the water to the flask.		
	5. Swirl the flask.	5a. To dissolve the potassium iodide.	
	6. Weigh 13 g of resublimed iodine, I_2.	6a. Use a trip balance.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)			
	7. Add it to the Erlenmeyer flask.		
	8. Swirl the flask.	8a. To dissolve the iodine.	
	9. Transfer the solution to a 1 liter volumetric flask.	9a. Rinse the Erlenmeyer flask with several small portions of water and add the rinsings to the volumetric flask.	
	10. Fill the flask to the 1 liter mark with water.		
	11. Thoroughly mix the contents of the flask.		
	12. Transfer the solution to a 1 liter glass-stoppered bottle.	12a. The strength of this solution is approximately 0.1N.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)			
5. Sodium Hydroxide, NaOH, 0.3N	1. Weigh 12 g of sodium hydroxide, NaOH.	1a. Use a trip balance.	
	2. Transfer it to a 2 liter Erlenmeyer flask.		
	3. Measure 1 liter of water.	3a. Use a 1 liter graduated cylinder.	
	4. Add the water to the flask.		
	5. Swirl the flask.	5a. To dissolve the sodium hydroxide.	
	6. Transfer the 0.3N base to a 1 liter plastic bottle.		
6. Hydrochloric Acid, HCl, 6 N	1. Measure 50 ml of water.	1a. Use a 100 ml graduated cylinder.	
	2. Pour it into a 250 ml Erlenmeyer flask.		
	3. Measure 50 ml of 12 N hydrochloric acid, HCl	3a. In a well ventilated area. 3b. Use a 100 ml graduated cylinder. The usual concentration of hydrochloric acid as it is purchased for ordinary laboratory use is 12N. More dilute concentrations can be purchased, however. Twelve N acid can be detected by gently blowing across the open bottle top, the formation of white fumes indicates that the hydrochloric acid is 12 N. The more dilute concentrations do not fume.	
	4. Pour it slowly into the flask.		
	5. Thoroughly mix the contents of the flask.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)			
	6. Transfer the 6N acid to a glass-stoppered bottle.		
7. Standard Phenylarsine oxide (PAO), 0.01N	1. Weigh 0.8 g of phenylarsine oxide (PAO) powder.	1a. Use a trip balance. 1b. Phenlarsine oxide (PAO) is extremely toxic. Wipe up any spilled powder with a damp tissue, discard the tissue, and thoroughly wash your hands with soap and water.	
	2. Transfer it to a 250 ml Erlenmeyer flask.		
	3. Measure 150 ml of 0.3N sodium hydroxide.	3a. Use a 100 ml graduated cylinder.	
	4. Pour it into the flask.		
	5. Stir the contents of the flask.	5a. By means of a magnetic stirrer. 5b. Until the PAO dissolves. It may take a long time.	
	6. Turn off the magnetic stirrer.		
	7. Allow any solid material remaining in the flask to settle.	7a. For 30 minutes.	
	8. Decant 110 ml of the supernatant liquid into a 250 ml graduated cylinder.		
	9. Measure 950 ml of water.	9a. Use a 1 liter graduated cylinder.	
	10. Pour it into a 2 liter Erlenmeyer flask.		

EFFLUENT MONITORING PROCEDURE: Titrimetric Determination of Total Residual Chlorine in Wastewater Effluents

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)			
	11. Place a mark at the 950 ml level.		
	12. Pour 150 ml of the water back into the graduated cylinder.		
	13. Discard the 150 ml of water.		
	14. Pour the 110 ml of supernatant PAO into the 800 ml of water in the 2 liter Erlenmeyer flask.		
	15. Swirl the flask.	15a. To thoroughly mix the contents.	
	16. Standardize a pH meter with a pH 7 buffer.		
	17. Measure the pH of the solution.	17a. It should be between 6.0 and 7.0. If it is not, do steps 18 through 20. If it is, proceed to step 22.	
	18. Add a few drops of the 6N hydrochloric acid to the flask.	18a. Use an eyedropper.	
	19. Swirl the flask.	19a. To thoroughly mix the contents.	
	20. Measure the pH of the solution.		
	21. Repeat steps 18 through 20 above until the pH of the solution is between 6.0 and 7.0.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Reagent Preparation (continued)			
	22. Add enough water to the flask to bring the level to the 950 ml mark.	22a. The PAO solution is also extremely toxic. If any is spilled on the skin, rinse it off immediately with large amounts of tap water.	
	23. Thoroughly mix the contents of the flask.		
	24. Transfer the solution to a 1 liter glass-stoppered bottle.		
8. Acetate Buffer Solution, pH 4.0	1. Measure 400 ml of water.	1a. Use a 500 ml graduated cylinder.	
	2. Pour it into a 1 liter volumetric flask.		
	3. Weigh 146 g of anhydrous sodium acetate, $NaC_2H_3O_2$.	3a. Use a trip balance. 3b. Two hundred forty-three g of sodium acetate trihydrate, $NaC_2H_3O_2 \cdot 3H_2O$ may also be used.	
	4. Transfer it to the flask.		
	5. Swirl the flask.	5a. To dissolve the solid.	
	6. Measure 457 ml of acetic acid, $HC_2H_3O_2$.	6a. Use a 500 ml graduated cylinder. 6b. In a well ventilated area.	
	7. Add it to the flask.		
	8. Add water to the 1 liter mark.		
	9. Swirl the flask.	9a. To thoroughly mix the contents.	
	10. Transfer the solution to a 1 liter glass-stoppered bottle.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Standardization of Reagents			
1. Standardization of the iodine.	1. Pipet 50.0 ml of the standard arsenite (B.3.) into a 250 ml Erlenmeyer Flask.	1a. Use a 50.0 ml volumetric pipet.	
	2. Measure 2 ml of starch; add it to the flask and mix.	2a. Use a 10 ml graduated cylinder.	
	3. Fill a 50 ml buret with the approximately 0.1N iodine (B.4.).	3a. Use a 10 ml graduated cylinder.	
	4. Remove air bubbles from the buret tip.		
	5. While swirling the flask (or using a magnetic stirrer), add iodine from the buret to the flask at a fast dropwise rate.	5a. Constant and thorough mixing is important.	
	6. When you see a blue color forming where the drops of iodine hit the liquid in the flask, stop the addition of the iodine.	6a. Even though a localized blue color formed, the overall solution should still be colorless when the solution is mixed. 6b. If it is blue, you have added too much iodine. Rinse out the flask and start again at step C.1.1. above.	
	7. Add 3 drops of concentrated hydrochloric acid to the flask.	7a. Use an eyedropper.	
	8. Swirl the flask.	8a. To thoroughly mix the contents. 8b. Bubbles of carbon dioxide will form.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Standardization of Reagents (continued)	9. While swirling the flask (or using a magnetic stirrer), continue addition of the iodine from the buret at a slower dropwise rate than before.	9a. About one-third as fast.	
	10. When the addition of 1 drop of the iodine causes formation of a permanent blue color, immediately stop the addition of the iodine.		
	11. Record the ml of iodine used.		
	12. Calculate the strength (normality, N) of the iodine.	12a. $N = \frac{C \times 50.0}{D}$ N = the strength (normality, N) of the iodine. C = the normality of the arsenic trioxide; B.3.27.27a. D = the ml of iodine used from the buret.	
2. Standard Iodine Titrant, 0.0282N.	1. Calculate the volume of iodine (B.4.) to be diluted to 1 liter to obtain a 0.0282N solution	1a. $E = \frac{F \times 1000}{G}$ E = ml of iodine (B.4.) to be diluted. F = desired N of the iodine after dilution, 0.0282N. 1000 = the desired volume (in ml) of the diluted iodine. G = N of the iodine to be diluted; (see C.1.12.12a.) 1b. $E = \frac{28.2}{G}$	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Standardization of Reagents (continued)			
	2. Measure 100 ml of water.	2a. Use a 100 ml graduated cylinder.	
	3. Pour it into a 1 liter volumetric flask.		
	4. Weigh 25 g of potassium iodide, KI.	4a. Use a trip balance.	
	5. Add the potassium iodide, KI, to the flask.		
	6. Swirl the flask.	6a. To dissolve the potassium iodide, KI.	
	7. Measure the calculated volume of iodine to be diluted to 1 liter to obtain a 0.0282 N solution.	7a. E will be an "unusual" volume, about 282 ml. Use a 500 ml graduated cylinder to measure to the nearest 10.0 ml, and a 10 ml graduated pipet to measure to the nearest 1.0 ml. For example, measure 280 ml in the cylinder, and 2.0 ml in the pipet.	
	8. Add it to the volumetric flask containing the potassium iodide solution.		
	9. Add water to the 1 liter mark.		
	10. Mix the contents of the flask thoroughly.		
	11. Transfer the 0.0282 N iodine solution to a 1 liter glass-stoppered bottle which has been wrapped in aluminum foil.	11a. The solution should be protected from the sunlight. 11b. This solution should be standardized on each day it is used. 11c. The standardization is carried out exactly as described in section C.1. above except: pipet 10.0 ml of arsenic trioxide instead of 50.0 ml, and use a 125 ml Erlenmeyer flask instead of a 250 ml flask. Also, the diluted iodine (0.0282 N) is used in the buret, instead of the approximately 0.1 N iodine.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Standardization of Reagents (continued)			
3. Standardization of the PAO	1. Pipet 10.0 ml of freshly standardized diluted iodine titrant (C.2.11.11c.) into a 125 ml Erlenmeyer flask.	1a. Use a 10 ml volumetric pipet.	
	2. Fill a buret (B.7) with the PAO.		
	3. Remove air bubbles from the buret tip.		
	4. Measure 1.0 ml of potassium iodide.	4a. Use a 10 ml graduated cylinder.	
	5. Add it to the flask.		
	6. Swirl the flask.	6a. To thoroughly mix the contents. 6b. The solution is red brown in color.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Standardization of Reagents (continued)			
	7. While swirling the flask (or using a magnetic stirrer), add PAO from the buret to the flask at a fast dropwise rate.	7a. Constant and thorough mixing is important.	
	8. When the color of the solution changes to a pale yellow color, stop the addition of PAO.		
	9. Measure 2.0 ml of starch.	9a. Use a 10 ml graduated cylinder.	
	10. Add the starch to the flask.		
	11. Swirl the flask.	11a. To thoroughly mix the contents. 11b. The solution is now medium to pale blue in color.	
	12. While swirling the flask, (or using a magnetic stirrer), continue addition of the PAO at a slower dropwise rate than before.	12a. About one-half as fast. 12b. At some point in the titration 1 drop of PAO will cause the solution to turn colorless. 12c. Immediately stop the addition. 12d. Record the ml of PAO used.	
	13. Calculate the strength (normality, N) of the PAO	13a. $N = \frac{H \times 10.0}{I}$ N = the strength (normality, N) of the PAO; it will be approximately 0.01. H = the normality of the iodine (C.2.11.11c.) I = the ml of PAO used from the buret	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Standardization of Reagents (continued)			
4. PAO Solution 0.00564N	1. Calculate the volume of PAO (C.3.13.13a.) to be diluted to 1 liter to obtain a 0.00564 N solution.	1a. $J = \frac{K \times 1000}{L}$ J = ml of PAO (C.3.13.13a.) to be diluted K = desired N of the PAO solution after dilution, 0.00564. 1000 = desired volume (in ml) of the diluted PAO L = N of PAO (C.3.13.13a.)	
	2. Measure the calculated volume of PAO to be diluted to 1 liter to obtain a 0.00564N solution.	2a. J will be an "unusual" volume; about 500 ml. If J is more than 500 ml, use a 1 liter graduated cylinder to measure to the nearest 10.0 ml. If J is less than 500 ml, use a 500 ml graduated cylinder to measure to the nearest 10.0 ml. After using the appropriate cylinder, use a 10 ml graduated pipet to measure to the nearest 1.0 or 0.1 ml.	
	3. Add the measured volume to a 1 liter volumetric flask.		
	4. Add water to the 1 liter mark.		
	5. Thoroughly mix the contents of the flask.	5a. If the dilution was done properly, the N of the PAO is 0.00564 N.	
5. Checking the N of the diluted PAO	1. Repeat steps C.3.1. through C.3.13.	1a. Except that the diluted PAO is added from the buret, instead of the stronger PAO. 1b. The N of the PAO is 0.00564. 1c. If it is not, discard the diluted PAO and repeat sections C.2., C.3., and C.4.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Procedure	1. Pipet 5.0 ml of the 0.00564 N PAO (C.S.) into a 30 ml beaker.	1a. Use a 5 ml volumetric pipet.	
	2. Weigh 1.0 g of potassium iodide, KI.	2a. Use a trip balance.	
	3. Add it to the beaker.		
	4. Stir the beaker contents.	4a. Use a stirring rod. 4b. To dissolve the solid.	
	5. Standardize a pH meter with a pH 4 buffer.		
	6. Check the pH of the solution in the beaker.	6a. It must be between 3.5 and 4.2 before the titration is begun. 6b. If it is, proceed to step 11. If it is not, do steps 7 through 10.	
	7. Add 5-10 drops of acetate buffer.	7a. Use an eyedropper.	
	8. Swirl the beaker.	8a. To thoroughly mix the contents.	
	9. Recheck the pH.	9a. It must be between 3.5 and 4.2 before the titration is begun.	
	10. Repeat steps 7, 8, and 9 above until the pH is between 3.5 and 4.2.		
	11. Pour the contents of the beaker into a 500 ml Erlenmeyer flask.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Procedure (continued)	12. Measure 200 ml of sample.	12a. There can be no delay between the time the sample is collected and the time the analysis is done. Protect the sample from sunlight and do not agitate it. 12b. Use a 250 ml graduated cylinder. 12c. Two hundred ml of sample are used when the expected concentration of total residual chlorine is less than 10 mg/l. 12d. One hundred fifty ml would be used if the expected concentration is between 10 and 15 mg/l. 12e. One hundred ml would be used if the expected concentration is between 15 and 20 mg/l.	
	13. Rinse the 30 ml beaker with several portions of sample from the graduated cylinder.	13a. Pour each rinse into the Erlenmeyer flask.	
	14. Pour the rest of the sample into the flask.		
	15. Swirl the flask.	15a. To thoroughly mix the contents.	
	16. Fill a 5 ml buret with the 0.0282 N iodine (C.2.11.11c.)		
	17. Remove air bubbles from the buret tip.		
	18. Measure 1.0 ml of starch.	18a. Use a 10 ml graduated cylinder.	
	19. Add it to the flask.		
	20. Swirl the flask.	20a. To thoroughly mix the contents.	
	21. While swirling the flask (or using a magnetic stirrer), add the iodine from the buret to the flask at a dropwise rate.	21a. About one drop per second. 21b. Thorough and constant mixing are essential during the titration.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Procedure (continued)	22. At some point the addition of one drop of iodine will cause formation of a blue color.	22a. The color will not fade on standing for a few seconds.	
	23. Immediately stop the addition of iodine when this happens.		
	24. Record the ml of iodine used from the buret.	24a. To the nearest 0.1 ml.	
E. Calculation	1. Calculate the total residual chlorine content of the sample in mg/l.	1a. mg of total residual chlorine per liter of sample = $\frac{(5.0 - 5\ A) \times 200}{B}$ 1b. A = ml of iodine used from the buret. B = ml of sample 1c. When B = 200, mg of total residual chlorine per liter of sample = (5.0 - 5 A).	

EFFLUENT MONITORING PROCEDURE: Titrimetric Determination of Total Residual Chlorine in Wastewater

TRAINING GUIDE

SECTION	TOPIC
I	Introduction
II	Educational Concepts - Mathematics
III	Educational Concepts - Science
IV	Educational Concepts - Communications
V*	Field & Laboratory Equipment
VI	Field & Laboratory Reagents
VII	Field & Laboratory Analysis
VIII	Safety
IX	Records & Reports

*Training guide materials are presented here under the headings marked *.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.1.1	If the glassware is especially dirty and cannot be cleaned with ordinary detergents, chromic acid cleaning may be required.	Standard Methods, 14th ed. 1975, p. 336, par. 2.c.2
	1. Pour 35 ml of distilled water in a 250 ml beaker.	
	2. Add about 1/8 teaspoon (simply estimate this quantity) of sodium dichromate, $Na_2Cr_2O_7$, to the water.	
	3. Swirl the beaker until the sodium dichromate has dissolved.	
	4. Keep repeating steps 2 and 3 until no more sodium dichromate will dissolve.	
	5. Pour the solution into a 2 liter beaker.	
	6. Slowly pour 1 liter of concentrated sulfuric acid, H_2SO_4, into the 2 liter beaker. CAUTION: Use eyeglasses and protective clothing.	
	7. Stir the mixture thoroughly.	
	8. Store it in a glass stoppered bottle.	
	9. The cleaning solution should be at a temperature of about 50°C when it is used.	
	10. It may therefore be necessary to warm the cleaning solution.	
	11. When using the warm cleaning solution, fill the piece of glassware with the solution.	
	12. Allow it to soak for 2-3 minutes (or longer).	
	13. Pour the cleaning solution back into the storage bottle.	
	14. Rinse the piece of glassware ten times with tap water.	
	15. The cleaning solution may be reused until it turns green.	
	16. It should then be discarded.	

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

DETERMINATION OF TOTAL SUSPENDED (NON-FILTERABLE) SOLIDS, mg/liter

as applied in

WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

Developed by the

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

EFFLUENT MONITORING PROCEDURE: Total Suspended (Non-Filterable) Solids, mg/liter

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EFFLUENT MONITORING PROCEDURE: Total Suspended (Non-Filterable) Solids, mg/liter

1. Objective:

To determine total suspended (non-filterable) solids on a weight (mg/liter) basis.

2. Brief Description of Analysis:

A well-mixed sample is filtered through a weighed, standard glass fiber filter disc in a filtration assembly. The filter disc with retained residue is dried in an oven at 103° - 105°C until a constant weight is obtained. The difference between the weight of the filter disc plus residue (g) and the original weight of the filter disc (g) is divided by the milliliters of sample filtered, then multiplied by 1,000,000. The final result is recorded as total suspended (non-filterable) solids, mg/liter.

3. Applicability of this Procedure:

a. Range of Concentration:

10 to 20,000 mg/liter

b. Pretreatment of Sample:

The Federal Register Guidelines do not specify any pretreatment.

c. Treatment of Interferences in Samples:

This procedure includes directions and information about choosing a sample volume small enough to prevent getting too much residue on the filter. (This entraps water and prolongs drying periods.)

No other interferences are noted in the Source of Procedure*.

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*Source of Procedure: Methods for Chemical Analysis of Water and Wastes, 1974, U.S. Environmental Protection Agency, Methods Development & Quality Assurance Research Laboratory, Cincinnati, Ohio, 45268, p. 268.

EFFLUENT MONITORING PROCEDURE: Total Suspended (Non-Filterable) Solids, mg/liter

Operating Procedures:

A. Prepare the filter disc

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60 minutes in oven at 103°-105°C
20-30 minutes in a desiccator

B. Prepare to test the sample
C. Weigh the filter disc
D. Seat the filter disc
E. Filter the sample
F. Wash down walls of filter apparatus
G. Dry filter disc and residue

J.1. Clean the filtration equipment

---

60 minutes in oven at 103°-105°C
20-30 minutes in a desiccator

H. Weigh filter disc and residue
I. Check for complete drying

---

30 minutes in oven at 103°-105°C
20-30 minutes in a desiccator

Finish check for complete drying

J.2 Clean filter disc support

K. Calculate total suspended (non-filterable) solids, mg/liter
L. Report the data

Equipment and Supply Requirements

A. Capital Equipment:

Balance, analytical, capable of weighing to 0.1 mg under a 200 g load

Oven, drying, for use at 103°-105°C.

Vacuum source or pump drawing 15 inches mercury

B. Reusable Supplies:

1 Cylinder, graduated, 25 or 50 ml

1 Cylinder, graduated, volume equal to or greater than the volume of sample to be filtered. (100 ml is commonly used. For sample volumes less than 10 ml, a wide-tip pipet can be used with a pipet bulb to draw sample into pipet.)

1 Desiccator (for storing filter discs on watch glasses, etc.)

1 Flask, suction, with side arm, 1000 ml

1 Hose connection from suction flask to vacuum source

1 Pinchcock clamp to use on hose

1 Filter holder: membrane filter holder assembly or Buchner funnel or Hirsch funnel. The filter holder should have a stopper which fits into the mouth of the 1000 ml suction flask. Gooch crucibles may be used--one for each sample plus one adapter to hold the crucibles in the mouth of the 1000 ml suction flask.

1 Support for filter disc during drying (watch glasses, etc., number depends on number of samples). If Gooch crucibles are used, omit this item.

1 Pair Tongs or gloves, etc., to remove crucibles or watch glass from the oven

1 Pair Forceps (flat, to handle filter discs)

1 Wash Bottle, squeeze type for distilled water

1 Set Cork Borers

C. Consumable Supplies:

Filter discs, glass fiber, without organic binder, Reeve Angel type 934A or 984H, Gelman type A, Whatman GF/C or equivalent. Diameter should be large enough so disc will cover openings in the filter holder to be used.

Marking ink to permanently mark glass or porcelain. A marking tool can be used instead.

Notebook, bound

Tissues, soft (for balance work)

Water, distilled

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
TOTAL SUSPENDED (NON-FILTERABLE) SOLIDS, mg/liter			I (p. 25)
A. Preparing the Filter Disc	1. Gather equipment.	1a. See page 6 for list of necessary equipment. The oven should be turned on and set for 103°-105°C temperature.	V.A.1b (p. 27)
		1b. Filter disc supports (watch glasses, etc.) or Gooch crucibles should have permanent identification marks.	V.A.1c (p. 27)
		1c. Be sure equipment is clean.	
	2. Place filter holder with stopper or adapter into the suction flask.	2a. Twist, pressing downward for air-tight fit.	
	3. Attach hose.	3a. From side arm of suction flask to the vacuum source.	
	4. Pick up a filter disc.	4a. Using forceps.	
	5. Place filter disc on the filter holder.	5a. Wrinkled surface of filter disc facing upward. 5b. Disc should cover all openings in filter holder.	
	6. Apply vacuum.	6a. Gradually, to seat the filter disc. A pinchcock clamp on the vacuum hose can be used to regulate application of vacuum. 6b. If a membrane filter holder is used, attach funnel now and tighten the collar.	
	7. Measure out about 20 ml distilled water.	7a. In a 25 or 50 ml graduated cylinder.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Preparing the Filter Disc (Continued)			
	8. Pour the 20 ml distilled water on to the disc.	8a. Vacuum still being applied. 8b. To rinse off the filter disc. 8c. If fibers of disc form a lumpy area, discard the disc and begin again at step 4.	
	9. Measure out about 20 ml distilled water.	9a. In the same graduated cylinder.	
	10. Pour this second 20 ml amount of distilled water on to the disc.	10a. Vacuum still being applied. 10b. A second rinse for the disc.	
	11. Measure out about 20 ml distilled water.	11a. In the same graduated cylinder.	
	12. Pour this third 20 ml amount of distilled water on to the disc.	12a. Vacuum still being applied. 12b. A third rinse for the disc.	
	13. Continue vacuum application.	13a. For 2 minutes to remove all traces of water. 13b. If a membrane filter holder is used, loosen the collar and remove the funnel.	
	14. Turn off vacuum.	14a. Break vacuum by pushing upward on the rubber adapter or stopper until air can enter the flask.	
	15. Loosen the filter disc from the filter holder.	15a. If a Gooch crucible is used, omit this step. 15b. If a membrane filter holder is used, use forceps to loosen the disc. Be careful not to damage the disc.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Preparing the Filter Disc (Continued)			
	16. Slide the filter disc onto a suitable support.	16a. If a Gooch crucible is used, remove the crucible with the filter disc in it. Wipe the outside with a tissue to remove droplets of water, fingerprints, etc. Do not directly handle the crucible during the procedure. Use tissue, forceps or tongs instead. 16b. If a membrane filter holder is used, use a dry watch glass, etc., to hold the disc. 16c. The filtration assembly can be left as is for future use.	
	17. Put disc (on support) into an oven.	17a. To dry at 103°-105°C. 17b. For 30 minutes in a mechanical convection oven. 17c. For 60 minutes in a gravity convection oven. 17d. Note: Do not open oven door during drying period.	
	18. Remove disc (on support) from oven.	18a. With tongs or gloves, etc.	
	19. Allow disc (on support) to cool partially to room temperature.	19a. Place on clean, heat-resistant surface for about three minutes.	
	20. Put disc (on support) into desiccator.	20a. With tongs or gloves, etc. 20b. Desiccant must be dry. 20c. Desiccator should be air-tight with enough room so disc supports do not touch each other or the side of the desiccator.	V.A.20a. (p. 27)
	21. Store disc in desiccator until needed.	21a. Disc and support should be cooled to room temperature before weighing--20 to 30 minutes.	

EFFLUENT MONITORING PROCEDURE: Total Suspended (Non-Filterable) Solids, mg/liter

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Preparing to Test the Sample	1. Assemble filtering equipment except for filter disc.	1a. Equipment list is on page 6. 1b. The filtering assembly used to prepare the disc (rinsing it) can be re-used at this time. 1c. Rinse water can remain in suction flask. 1d. Filter holder or Gooch crucible adapter should be tightly in mouth of suction flask. 1e. The oven should be turned on and set for 103°-105°C temperature.	
	2. Record the sample identification information.	2a. Sample should be at hand before continuing with this test. 2b. Use a laboratory notebook. 2c. Record "identification", "type", "date and time collected", and name of "sample collector."	VII.B.2a (p. 28) IX.B.2b (p. 31) IX.B.2c (p. 31)
C. Weighing the Filter Disc	1. Bring forceps, record book and pen to balance table.	1a. Use an analytical balance.	
	2. Zero the balance.		
	3. Remove filter disc (on support) from desiccator.	3a. If a Gooch crucible is being used, use a tissue, forceps or tongs to remove it from the desiccator. It should contain a rinsed, dried filter disc.	
	4. Record filter disc identification.	4a. Gooch crucible number or watch glass number. (Examples: C-12, WG-1) 4b. In laboratory notebook. 4c. In column of the sample for which this disc will be used. 4d. Labeled "filter identification."	V.C.4a (p. 27) IX.C.4 (p. 32)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Weighing the Filter Disc (Continued)			
	5. Place filter disc on balance pan.	5a. If a Gooch crucible is being used, use a tissue, forceps or tongs to place it on the balance pan. 5b. If a membrane filter holder is being used, use forceps to slide the filter disc from the storage support (watch glass, etc.) on to the pan.	
	6. Weigh the filter disc.	6a. To four decimal places. 6b. For Gooch crucibles, you can save weighing time by keeping a list of the numbered crucibles with their approximate weights so you have a beginning weight for this operation.	
	7. Record the weight.	7a. In laboratory notebook, 7b. In column of the sample for which this disc will be used. 7c. Labeled "weight of filter (g)." If Gooch crucibles are used, this is the weight of the crucible containing a filter disc.	IX.C.7 (p. 32)
	8. Remove the filter disc from the balance pan.	8a. If a Gooch crucible is being used, use tissue, forceps or tongs to remove crucible containing filter disc. 8b. If a membrane filter holder is being used, use forceps to slide the filter disc from the pan on to its storage support (watch glass, etc.).	
	9 Return all weights on the balance to zero position.		

EFFLUENT MONITORING PROCEDURE: Total Suspended (Non-Filterable) Solids, mg/liter

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. Seating the Filter Disc	1. Slide the filter disc on to the filter holder held in the mouth of the suction flask.	1a. If a Gooch crucible is used, put crucible and disc into the Gooch crucible adapter. 1b. If a membrane filter holder is used, place the wrinkled surface of the disc facing upward on the filter holder. 1c. If a series of funnel type filter assemblies are used, be sure to write the filter disc identification number on the corresponding funnel or flask.	
	2. Apply vacuum.	2a. Gradually, to help seat filter disc. A pinchcock clamp on the vacuum hose can be used to regulate application of vacuum. 2b. If a membrane filter holder is being used, attach funnel now and tighten the collar.	
	3. Pour about 5 ml distilled water on to the filter disc.	3a. Vacuum still being applied. 3b. Can use squeeze bottle of distilled water and estimate volume. 3c. Wetting helps seat filter against holder.	
	4. Leave vacuum on.		
E. Filtering the Sample	1. Record date and time.	1a. In laboratory notebook. 1b. In column of the sample to be filtered. 1c. Labeled "Date and Time Analysis Began."	IX.E.1 (p. 32)
	2. Select the volume of sample to be filtered.	2a. 100 ml of sample is a commonly used volume. 2b. CAUTION: Too much residue on the filter will entrap water and may require prolonged drying. If suspended solid concentration in the sample is obviously great, choose a less-than-100 ml volume of well mixed sample.	VII.E.2b (p. 29)
	3. Shake the sample.	3a. So portion used is representative of all the sample.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. Filtering the Sample (Continued)	4. Immediately measure out the selected volume.	4a. Using a graduated cylinder (use a wide tip pipet for volumes less than 10 ml). 4b. Measure rapidly since solids may settle in the sample container while you are filling the cylinder. 4c. If you pour the sample to above the graduations, pour that sample back into the bottle and begin again at Step 3.	
	5. Pour the sample on to the filter disc in the filtration assembly.	5a. You should filter all the sample you measure out because you should rinse remaining, settled solids out of the cylinder and on to the filter disc. 5b. If a series of samples are being filtered, be sure you filter each sample through the filter disc you weighed and designated for that sample on the lab data sheet.	
	6. Rinse any sample left in the cylinder on to the filter disc.	6a. With distilled water. 6b. As required. 6c. If suspended solid concentration on the filter disc is obviously small, measure additional volumes of well-mixed sample and filter these, rinsing the cylinder each time.	
	7. Leave suction on.		
	8. Record the total ml of sample filtered.	8a. In laboratory notebook. 8b. In column of the sample filtered. 8c. Labeled "ml Sample Filtered."	IX.E.8 (p. 32)

EFFLUENT MONITORING PROCEDURE: Total Suspended (Non-Filterable) Solids, mg/liter

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Washing Walls of Filter Apparatus	1. Rinse walls of filter holder with about 10 ml distilled water.	1a. A squeeze bottle of distilled water can be used. Estimate the 10 ml volume. 1b. Otherwise, use a graduate and direct the rinse onto the walls. 1c. Suction should be applied.	
	2. Allow time for complete drainage.		
	3. Rinse walls of filter holder with another 10 ml distilled water.	3a. See information above for F.1.	
	4. Allow time for complete drainage.		
	5. Rinse walls a third time with about 10 ml distilled water.	5a. See information above for F.1. 5b. NOTE: The diluted filtrate cannot be used for a dissolved solids determination.	
	6. Continue vacuum application.	6a. For two minutes to remove all traces of water. 6b. If a membrane filter holder is used, loosen the collar and remove the funnel.	
	7. Turn off vacuum.	7a. Break vacuum by pushing upward on the rubber adapter or stopper until air can enter the flask.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
G. Drying the Filter Disc and Residue	1. Loosen the filter disc from the filter holder.	1a. If a Gooch crucible is used, omit this step. 1b. If a membrane filter holder is used, use forceps to loosen the filter disc.	
	2. Slide the filter disc plus residue on to its support.	2a. If a Gooch crucible is used, remove the crucible with the filter disc in it. Wipe the outside with a tissue to remove droplets of water, fingerprints, etc. before drying. 2b. If a membrane filter holder is used, slide the filter disc on to the same marked watch glass you used earlier for its support.	
	3. Put disc (on support) into an oven.	3a. To dry at 103°-105°C. 3b. For 30 minutes in a mechanical convection oven. 3c. For 60 minutes in a gravity convection oven. 3d. NOTE: Do not open oven door during drying period. 3e. NOTE: While solids are in drying oven, do "J. Cleaning the Equipment, Step 1."	VII.G.3b (p. 29)
	4. Remove disc (on support) from oven.	4a. With tongs or gloves, etc. 4b. Let oven turned on and set for 103°-105°C temperature.	
	5. Allow disc (on support) to cool partially to room temperature.	5a. Place on clean, heat-resistant surface for about three minutes.	
	6. Put disc (on support) into desiccator.	6a. Desiccant must be dry. 6b. Desiccator should be air-tight with enough room so disc supports do not touch each other or the side of the desiccator.	V.G.6a (p. 27)
	7. Allow time for disc to cool to room temperature.	7a. Twenty to 30 minutes.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
H. Weighing the Filter Disc and Residue			
	1. Bring forceps, record book and pen to balance table.	1a. Use the same analytical balance you used earlier to weigh the disc.	
	2. Zero the balance.		
	3. Remove filter disc plus residue (on support) from desiccator.	3a. If a Gooch crucible is being used, use a tissue, forceps or tongs to remove it from the desiccator.	
	4. Place filter disc on balance pan.	4a. If a Gooch crucible is being used, use a tissue, forceps or tongs to place it on the balance pan. 4b. If a membrane filter holder is being used, use forceps to slide the filter disc from the storage support (watch glass, etc.) onto the pan.	
	5. Weigh the filter disc plus residue.	5a. To four decimal places. 5b. Use the "weight of the filter" (or of the Gooch crucible with filter) on your Laboratory Data Sheet as a beginning weight.	
	6. Record the weight.	6a. In laboratory notebook, 6b. In column of the sample for which the disc was used. 6c. Labeled "1st weight of filter plus residue (g)." If Gooch crucibles are used, this is the weight of the crucible containing a filter disc with residue.	IX.H.6 (p. 32)
	7. Remove the filter disc from the balance pan.	7a. If a Gooch crucible is being used, remove crucible containing filter disc with residue. 7b. If a membrane filter holder is being used, use forceps to slide the filter disc with residue back on to its support (watch glass, etc.)	
	8. Return all weights on the balance to zero position.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
I. Check for Complete Drying	1. Put disc plus residue (on support) into an oven.	1a. At 103°-105°C 1b. For 30 minutes. 1c. NOTE: Do not open oven door during drying period.	VII.I (p. 30)
	2. Remove disc (on support) from oven.	2a. Using tongs or gloves, etc. 2b. Let oven turned on and set for 103°-105°C temperature.	
	3. Allow disc (on support) to cool partially to room temperature.	3a. Place on clean, heat-resistant surface for about three minutes.	
	4. Put disc (on support) into desiccator.	4a. Desiccant must be dry. 4b. Desiccator should be air-tight with enough room so disc supports do not touch each other or the side of the desiccator.	
	5. Allow time for disc to cool to room temperature.	5a. Twenty to 30 minutes.	
	6. Bring forceps, record book and pen to balance table.	6a. Use an analytical balance.	
	7. Zero the balance.		
	8. Remove filter disc (on support) from desiccator.	8a. If a Gooch crucible is being used, use a tissue, forceps or tongs to remove it from the desiccator.	
	9. Place filter disc on balance pan.	9a. If a Gooch crucible is being used, use a tissue, forceps or tongs to place it on the balance pan. 9b. If a membrane filter holder is being used, use forceps to slide the filter disc from the storage support (watch glass, etc.) on to the pan.	
	10. Weigh the filter disc plus residue.	10a. To four decimal places. 10b. Use the "1st weight of the filter plus residue (g)" recorded on your data sheet for this sample as a beginning weight.	

EFFLUENT MONITORING PROCEDURE: Total Suspended (Non-Filterable) Solids, mg/liter

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
I. Check for Complete Drying (Continued)	11. Record the weight.	11a. In laboratory notebook. 11b. In column of the sample for which the disc was used. 11c. Labeled "2nd weight of filter plus residue (g)." If Gooch crucibles are used, this is the weight of the crucible containing a filter disc with residue.	IX.I.11 (p. 32)
	12. Remove the filter disc plus residue from the balance pan.	12a. If a Gooch crucible is being used, remove crucible containing disc with residue. Save this. 12b. If a membrane filter holder is being used, use forceps to slide the filter disc with residue back on to its support (watch glass, etc.). Save this.	
	13. Return all weights on the balance to zero position.		
	14. Find the difference between the 1st and 2nd weights of the filter plus residue.	14a. In laboratory notebook. 14b. In column of the sample for which the disc was used. 14c. Labeled "difference (1st-2nd)."	IX.I.14 (p. 32)
	15. Inspect the difference for acceptable agreement of these two weights.	15a. If the weights agree, drying was complete so the procedure is finished. 1) The weights should ideally be constant [the same weight, ± the possible balance error of 0.0001g (0.1 mg)]. Use the last weight obtained. 2) An acceptable difference between these successive weights is no more than 0.0005g (0.5 mg). In this case, use the last weight obtained for the "final weight of filter plus residue (g)" on line 13 of the Laboratory Data Sheet. (Continued)	II.I.15a.1 (p. 26) II.I.15a.2 (p. 26)

EFFLUENT MONITORING PROCEDURE: Total Suspended (Non-Filterable) Solids, mg/liter

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
I. Check for Complete Drying (Continued)		15b. If the weights do not meet the requirements of agreement, repeat this "Section I: Check for Complete Drying" until you do obtain two successive weights that agree according to a.1) or a.2) above. Use the last weight obtained as the "final weight of filter plus residue (g)" on line 13 of the Laboratory Data Sheet.	IX.I.15 (p. 32)
	16. Sign the laboratory data sheet.	16a. In laboratory notebook. 16b. In column for sample(s) you tested. 16c. Labeled "analyst."	IX.I.16 (p. 32)
	17. Turn oven off.		
	18. Discard the filter disc plus residue.	18a. Unless there is some reason for saving the solids. 18b. The filter disc support should be cleaned according to "J. Cleaning the Equipment, Step 2."	
J. Cleaning the Equipment	1. Clean the filtration equipment as soon as possible after use (See G.3e).	1a. Membrane filter holder assembly: Leave disc support in suction flask, use squeeze bottle of distilled water, rinse disc support while applying gentle suction. Assembly need not be completely dry before re-use. 1b. Hirsch funnel or Buchner funnel: Leave funnel in suction flask and rinse with distilled water as described above in J.1.1a. 1c. Gooch adapter: Leave in suction flask and rinse the small glass funnel with distilled water (squeeze bottle) while applying gentle suction. Adapter need not be completely dry before re-use. 1d. Suction flask: Remove the rinsed filter holder. Empty the flask through the top (not the side-arm). Rinse it with tap water. Flask need not be completely dry before re-use. (Continued)	

EFFLUENT MONITORING PROCEDURE: Total Suspended (Non-Filterable) Solids, mg/liter

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
J. Cleaning the Equipment (Continued)		1e. Graduated cylinders: Rinse with distilled water. These should be dry before re-use.	
		1f. If stronger cleaning measures are required, use directions given in the Training Guide.	V.J.1f (p. 27)
	2. Clean the filter disc support as soon as possible after use. (See I.17.b)	2a. Gooch crucibles: Rinse with distilled water and shake off excess. Crucible need not be completely dry before re-use. 2b. Disc support (watch glass etc.): Rinse with distilled water. Dry completely before re-use.	
		2c. If stronger cleaning measures are required, use directions given in Training Guide.	V.J.2c (p. 27)
K. Calculations	1. Use the following steps to calculate total suspended (non-filterable) solids, mg/liter.	1a. The calculation formula is: Total suspended solids, mg/liter = $\frac{[(\text{g.wt. filter plus residue})\text{minus}(\text{g.wt. filter})] \times 1000 \times 1000}{\text{ml sample filtered}}$	
		1b. The "Typical Laboratory Data Sheet" has the steps and an example for doing this calculation.	IX. (p. 32)
		1c. Numbers used in the examples below are from the example in the third "Sample" column on the "Typical Laboratory Data Sheet."	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
K. Calculations (Continued)			
	2. Subtract the "weight of filter (g)" on line 14 from the "final weight of filter plus residue (g)" on line 13.	2a. Example on data sheet: line 13 - 0.1413 g. line 14 - 0.1293 g. Difference = 0.0120 g.	
		2b. NOTE: This is the gram weight of the residue which was on the filter disc.	
		2c. IMPORTANT: This gram weight of the residue (the difference) should be greater than 0.0025 g. If the weight of the residue (the difference) is less than 0.0025 g, you should repeat the procedure and filter a larger volume of the sample so more residue is obtained.	VII.K.2c. (p. 30)
	3. Write the difference on line 15 of the data sheet.	3a. This has been done for the example in the third "Sample" column.	IX.K.3 (p. 32)
	4. Divide this difference on line 15 by the "ml sample filtered" on line 7 to get a 7 decimal place answer.	4a. Example on data sheet: $\frac{\text{line 15}}{\text{line 7}} = \frac{0.0120\text{g}}{67.0\text{ ml}} = 0.0001791$ g/ml	
		4b. NOTE: This is the gram weight of residue in each ml of the sample.	
	5. Write this answer on line 16.	5a. This has been done for the example in the third "Sample" column	IX.K.5 (p. 32)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
K. Calculations (Continued)			
	6. Multiply the 7 decimal place answer on line 16 by 1,000,000 (Move the decimal point 6 places to the right).	6a. Example on data sheet: line 16 is 0.0001791 g/ml x 1,000,000 = 179.1 mg/liter 6b. NOTE: This multiplication converts the gram weight of residue per ml to the unit of mg/liter.	
	7. Write this answer on line 17.	7a. This has been done for the example in the third "Sample" column.	IX.K.7 (p. 32)
	8. Round off answer on line 17 to to the nearest whole mg.	8a. Example on data sheet: line 17, 179.1 mg/liter becomes: 179 mg/liter	II.K.8a (p. 26)
	9. Write this answer on line 18.	9a. This has been done for the example in the third "Sample" column. 9b. Records should be kept in a laboratory notebook.	IX.K.9 (p. 32)
L. Reporting Data	1. Report total suspended (non-filterable) solids, mg/liter.	1a. On any required record or report sheets.	IX.L.1a (p. 31)

TRAINING GUIDE

SECTION	TOPIC
*I	Introduction
*II	Educational Concepts-Mathematics
III	Educational Concepts-Science
IV	Educational Concepts-Communications
*V	Field & Laboratory Equipment
VI	Field & Laboratory Reagents
*VII	Field & Laboratory Analysis
VIII	Safety
*IX	Records & Reports

*Only these sections are used in this procedure.

EFFLUENT MONITORING PROCEDURE: Determination of Total Suspended (Non-Filterable) Solids, mg/liter

INTRODUCTION

Section I

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
	Suspended solids are insoluble solids that are in suspension or dispersed in water, wastewater, or other liquids. These are largely removable by standard filtering procedures in a laboratory.	Glossary Water & Wastewater Control Engineering. 1969. WPCF, Washington, DC 20016
	The term "suspended solids" is used here to refer to the quantity of material removed from wastewater under specified laboratory test conditions. The test described in this instruction can be found in the EPA Methods Manual on page 268, entitled "Residue, Total Non-Filterable."	Methods for Chemical Analysis of Water and Wastes. 1974. EPA-MDQARL, Cincinnati, OH 45268, p. 268
	The amount of suspended solids in samples can be used to indicate the efficiency of primary and final settling tanks and the quality of plant effluent. Thus the results of this test are used for plant control and for regulatory requirements.	
	This procedure to determine suspended solids can also be found in Standard Methods on page 94, entitled "Total Nonfiltrable Residue Dried at 103-105C (Total Suspended Matter)."	Standard Methods for the Examination of Water and Wastewater. 14th ed., 1976. APHA, Washington, DC. p. 94

TRAINING GUIDE NOTE | REFERENCES/RESOURCES

I.15a.1 EXAMPLE of constant weights that differ only by a possible balance error of ± 0.0001g (0.1 mg)

True weight = 0.1286g

1st wt. obtained = 0.1287g (True + 0.1 mg)
2nd wt. obtained = 0.1285g (True - 0.1 mg)
Difference = 0.0002g

Thus to agree within possible balance error, the difference between the two weights should not be more than 0.0002g (0.2 mg).

I.15a.2 EXAMPLE of an acceptable difference between successive weights where the difference is not more than 0.0005g (0.5 mg):

1st wt. obtained = 0.1287g
2nd wt. obtained = 0.1283g
Difference = 0.0004g (0.4 mg)

Use the 2nd wt. obtained.

K.8a. Rounding results to the nearest whole mg: If the digit 0,1,2,3 or 4 is dropped, the preceding digit is not altered.

EXAMPLE: 10.4 mg is rounded to 10 mg

If the digit 5 is dropped, the preceding digit is rounded off to the nearest even number.

EXAMPLES: 10.5 mg is rounded to 10 mg
11.5 mg is rounded to 12 mg

If the digit 6,7,8 or 9 is dropped, the preceding digit is increased by one unit.

EXAMPLE: 10.6 mg is rounded to 11 mg

Standard Methods for the Examination of Water and Wastewater. 14th ed., 1976. APHA, Washington, DC. p. 18

U.S. EPA, Handbook for Analytical Quality Control in Water and Wastewater Laboratories. 1972, EPA-AQCL, Cincinnati, OH 45268. p. 7-2

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.1b. C.4a.	Gooch crucibles or filter disc supports (watch glasses, etc.) should have identification marks which will not be lost at the oven temperature of 103°-105°C. Gooch crucibles with this type marking can be purchased from laboratory supply companies. You can permanently mark glass or porcelain surfaces with an electrical marking tool or with marking ink followed by firing in a flame. You can purchase the tool or ink, or you can make marking solutions of ferric chloride or of ordinary blue-black ink fortified with a few grams of dissolved iron-potassium tartrate. The marks are melted onto the surface by firing in a flame or oven.	Hamilton and Simpson, Quantitative Chemical Analysis. 1958. Macmillan, NY, NY p. 40
A.1c. J.1f. J.2c.	The suction flask does not require cleaning. Using a soft brush, clean all other equipment with soap or detergent. If stronger cleaning measures are required, soak equipment in dilute acid or chromic acid cleaning mixture.	U.S. EPA, Handbook for Analytical Quality Control in Water and Wastewater Laboratories. 1972. EPA-AQCL, Cincinnati, OH 45268, p. 4-7
	After cleaning, rinse the equipment three times with tap water and three times with distilled water.	
	The following do not have to be completely dry before using: Gooch crucibles, filter funnels, filter holders, suction flasks.	
	The following should be completely dry before using: graduated cylinders for measuring samples, filter supports such as watch glasses.	
A.20a. G.6a.	Desiccants are hygroscopic materials capable of absorbing moisture from air. Silica gel (SiO_2) and calcium sulfate ($CaSO_4$) are two commonly used desiccants available from laboratory supply companies. These change color as they become saturated. The moisture can be removed from the desiccant by heating it in an oven.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
B.2a.	COLLECTION OF SAMPLES FOR THIS TEST: Samples should be collected from a preagreed site by a preagreed technique known to all parties concerned. You should be familiar with the following information since you record most of it on your laboratory data sheet. You may be responsible for actually collecting the sample; consult your supervisor.	
	LOCATION - Plant control and self-monitoring requirements will be the basis for selecting places to collect samples. Final collection points should be such that samples drawn there are as representative of the entire sample source as possible. Consult your supervisor.	Standard Methods for the Examination of Water and Wastewater. 14th ed., 1976, APHA, Washington, DC, p. 38
	IDENTIFICATION - Each collection location should be assigned a number or simple identification code. Use this to label samples from that location and to record on the lab data sheet.	
	TYPE - Permit requirements determine whether a grab or a composite sample will be collected; consult your supervisor. Mark type on sample container and on laboratory data sheet.	Ibid., p. 40
	TIME OF COLLECTION - Mark time and date on sample container and on lab data sheet.	
	CONTAINER - The analyst should know what volume container is required for each sample source. Containers should be capped, of resistant (to adsorption of solids) glass or plastic. Clean used containers by rinsing with dilute hydrochloric acid solution, with tap water (3 rinses) and with distilled water (3 rinses). Shake out excess water.	Methods for Chemical Analysis of Water and Wastes. 1974. EPA-MDQARL, Cincinnati, OH 45268, p. xi
	COLLECTION - Rinse container two or three times with sample, then collect the sample. Consult the analyst about the volume required from each sample source. Exclude very large solids like leaves, sticks, fish, lumps of fecal matter, etc. Put cap on container.	Ibid., p. 268

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
	SIGNATURE - Sample Collector should sign name on container or label so this information can be recorded on the lab data sheet.	
	STORAGE - It is not practical to preserve and store these samples. Analyze promptly to minimize chemical and/or physical changes.	
E.2b.	You want to filter a volume of sample such that prolonged drying times are not required (up to 0.200 g) *but* that will yield a significant weight of residue (at least 0.0025 g) on the filter disc.	
	Experience with samples from the same locations will help you choose such volumes.	
	One useful guide (except for samples containing a very high concentration of suspended matter, or which filter very slowly) is to select a sample volume of 14 ml or more per square cm of filter area. (Recall that for a circle, area = 3.14 times radius squared.)	Standard Methods for the Examination of Water and Wastewater. 13th ed., 1971. APHA, New York, NY p. 537
	You can also use turbidity to estimate sample size. If the sample has a turbidity of 50 units or less, filter a liter of sample. For turbidity greater than 50 units, filter sufficient sample to yield up to 50 mg and not more than 100 mg of residue. (If you are using a Gooch crucible, 50 mg is the practical limit due to drying requirements.)	*Ibid*, p. 291
G.3b.	The time required for *complete* drying depends on the amount and nature of the solids on the filter disc. The drying time given in this procedure is the *MINIMUM* time to be used.	
	1. If the solids have a glassy, wet appearance after the MINIMUM drying time, increase this drying time.	
	If you routinely run this test on samples from the same source and check them for complete drying (see I. in the procedure), you could choose a smaller sample volume for future determinations so that a longer drying time will not be necessary.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
I.	The need to verify the complete drying of the filter plus residue is important enough to warrant the extra time required to make this check. The weight of traces of water allowed to remain in the residue would contribute significant error to the final results in this test. The check for complete drying presented in this section depends on obtaining a constant (same) weight after repeating the heating, cooling and weighing cycle for the filter plus residue.	Methods for Chemical Analysis of Water and Wastes. 1974. EPA-MDQARL, Cincinnati, OH 45268, p. 269
K.2c.	If the weight of the residue is less than 0.0025g, there is not enough weight to be significant for this direct weighing method.	Standard Methods for the Examination of Water and Wastewater. 13th ed., 1971. APHA, New York, NY, p. 291

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
B.2b.	All laboratory records must be kept for three years, preferably in a permanently bound notebook. The time period is required by regulatory agencies.	
B.2c.	Attached as the next page is a typical laboratory data sheet for recording weights and for the later calculation of final results for suspended solids determinations.	
L.1a.	Depending on your organizational set-up, it may be your job responsibility to enter this data on the plant operation record, state report form, etc. Check with your supervisor.	

Typical Laboratory Data Sheet

for

TOTAL SUSPENDED (NON-FILTERABLE) SOLIDS, mg/liter

Name of Plant ______________________________

STEP	SUSPENDED SOLIDS	SAMPLE	SAMPLE	SAMPLE	
B.2	Identification			INS #1	1
B.2	Type (grab, etc.)			GRAB	2
B.2	Date & Time Collected			5/1/74 0900	3
B.2	Sample Collector			Tom Sampler	4
C.4	Filter Identification			WG2	5
E.1	Date & Time Analysis began			5/1/74 1100	6
E.8	ml Sample Filtered			67.0	7
H.6	1st weight of Filter* plus Residue (g)			0.1426	8
I.11	2nd weight of Filter* plus Residue (g)			0.1416	9
I.14	Difference (1st-2nd)			0.0010	
I.15	3rd weight of Filter* plus Residue (g)			0.1413	11
I.15	Difference (2nd-3rd)			0.0003	12
I.15	Final weight of Filter* plus Residue (g)			0.1413	13
C.7	Weight of Filter* (g)			0.1293	14
K.3	Find Difference (g) by subtracting Line 14 from Line 13			0.0120	15
K.5	Divide to 7 decimal places: $\frac{\text{(line 15) difference (g)}}{\text{(line 7) ml sample filtered}}$			0.0001791	16
K.7	Multiply Line 16 by 1000 000 (move decimal point 6 places Rt.)			179.1	17
K.9	Round answer on Line 17 to nearest whole number			179 mg/1	18
I.16	Analyst			Mary Analyst	19

*"Filter" means the filter disc if a funnel type filtration assembly is used. If Gooch crucibles are used "filter" means the crucible containing a filter disc.

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for

SETTLEABLE SOLIDS, ml/liter
(IMHOFF SETTLING CONE)

as applied in
WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

Developed by the

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

EFFLUENT MONITORING PROCEDURE: Settleable Solids, ml/liter (Imhoff Settling Cone)

This operational procedure was developed by:

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POSITION Chemist-Instructor

EDUCATION AND TECHNICAL BACKGROUND

B.A. - Edgecliff College

1 year Industrial Research Chemist

8 years Secondary School Chemistry Instructor

4 years DHEW-DI Water Quality Program Chemist

7 1/2 years DI-EPA Chemist-Instructor

EFFLUENT MONITORING PROCEDURE: Settleable Solids, ml/liter (Imhoff Settling Cone)

1. Analysis Objective:

To determine settleable matter on a volume (ml/l) basis.

2. Brief Description of Procedure:

A one liter sample is poured into an Imhoff Cone and the volume (ml/l) of settleable solids is recorded after a one hour settling period.

3. Applicability of this Procedure:

a. Range of Concentration:

The one Source of Procedure* cited in the Federal Register Guidelines does not state a range of concentration. This EMP includes a procedure for cases when the settleable solids exceed the graduations on an Imhoff Cone.

b. Pretreatment of Samples:

The Federal Register Guidelines do not specify any pretreatment, nor does the Source of Procedure*.

c. Treatment of Interferences in Samples:

The Source of Procedure does not note any interferences to this determination.

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*Source of Procedure: Standard Methods for the Examination of Water and Wastewater, 14th ed., APHA, Washington, D.C., p. 95.

EFFLUENT MONITORING PROCEDURE: Settleable Solids, ml/liter (Imhoff Settling Cone)

Flow Sheet for Determination:

1. Mix sample
2. Fill cone
3. Settle 45 minutes
4. Stir gently or swirl
5. Settle 15 minutes
6. Read results

EFFLUENT MONITORING PROCEDURE: Settleable Solids, mg/liter (Imhoff Settling Cone)

Equipment and Supply Requirements:

A. Capital Equipment: None

B. Reusable Supplies:

1. Imhoff Settling Cone, glass or plastic, with or without stopcock, graduated to 40 ml and with a graduation at 1 liter volume
2. Imhoff Cone support, 3 place
3. Imhoff Cone brush (or centrifuge tube brush)
4. Stirring rod, the same length as cone
5. Timer, interval, 60 minute minimum with alarm

C. Consumable: None

EFFLUENT MONITORING PROCEDURE: Settleable Solids, ml/liter (Imhoff Settling Cone)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
SETTLEABLE SOLIDS			I (p. 11)
A. Preparation for the Determination	1. Gather equipment for determination.	1a. Imhoff cone 1b. Imhoff cone support 1c. Long stirring rod 1d. Timer	
	2. Clean the cone and rod.	2a. If required 2b. Water drains without leaving many droplets.	V.A.2b. (p. 12)
	3. Bring sample to work area.	3a. Sample should be at hand before continuing with this test.	VII. A.3a. (p. 13)
	4. Record sample identification.	4a. In laboratory notebook 4b. In column to be used for sample 4c. Identification, type, date and time collected, sample collector.	IX.A.4a. (p. 15) IX.A.4c. (p. 15)
	5. Record cone identification information.	5a. If more than one cone is to be in use 5b. Cones can be numbered with a lab marking pen or pencil. 5c. Record identification in laboratory notebook 5d. In column-for corresponding sample 5e. Labeled "Cone Identification"	IX.A.5. (p. 16)
B. Determination	1. Record date and time.	1a. In laboratory notebook 1b. In column to be used for sample 1c. Labeled "Date & Time Analysis Began"	IX.B.1. (p. 16)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Determination (Continued)	2. Thoroughly mix the sample.	2a. Use a stirring rod or swirl gently. 2b. Do not shake the sample.	VII.B.2b. (p. 14)
	3. Fill the Imhoff Cone to the 1 liter mark with sample.		
	4. Set timer at 45 min.		
	5. Allow the sample to settle 45 minutes,	5a. in quiescent location 5b. not in sunlight	VII.B.5a. (p. 14) VII.B.5b. (p. 14)
	6. Gently stir the sample along the inner wall of the cone,	6a. to dislodge solids clinging to inner wall. 6b. Use a long stirring rod or else spin the cone.	
	7. Set timer at 15 minutes.		
	8. Allow the sample to settle for 15 more minutes.		
C. Results	1. Read the final volume of settled solids,	1a. in ml 1b. with eye at level of surface of settled matter. 1c. In rare cases, the final volume of settled matter may be above the 40 ml mark	VII.C.1c. (p. 14)
	2. Record the final volume of settled solids in the cone	2a. in laboratory notebook, 2b. in column used for that sample, 2c. labeled "Final Volume of Settleable Solids, ml/liter", 2d. to nearest whole ml per liter.	IX.C.2a. (p. 15) IX.C.2. (p. 16)

EFFLUENT MONITORING PROCEDURE: Settleable Solids, ml/liter (Imhoff Settling Cone)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Results(Continued)	3. Sign the laboratory data sheet.	3a. Labeled "Analyst"	IX.C.3. (p. 16)
	4. Report Settleable Solids, ml/liter	4a. on any required record sheets.	IX.C.4a. (p. 15)
D. Cleaning Equipment	1. Discard cone contents.		
	2. Rinse the cone and stirring rod	2a. with tap water	V.D.2a. (p. 12)
	3. Complete cleaning the cone and rod as soon as possible,	3a. to prevent algal growth	V.D.3a. (p. 12)

524

525

TRAINING GUIDE

SECTION	TOPIC
*I	Introduction
II	Educational Concepts-Mathematics
III	Educational Concepts-Science
IV	Educational Concepts-Communications
*V	Field & Laboratory Equipment
VI	Field & Laboratory Reagents
*VII	Field & Laboratory Analyses
VIII	Safety
*IX	Records & Reports

*Only these sections are used in this procedure.

INTRODUCTION

Section I

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
	Settleable solids would be that matter in wastewater which will not stay in suspension during a preselected settling period (such as one hour) but either settles to the bottom or floats to the top. In the Imhoff Cone test, the settling period is one hour and the quantity of solids is expressed by volume (ml/l) of settled matter.	Glossary Water and Wastewater Control Engineering. 1969. WPCF, Washington, DC 20016.
	The test described in this instruction is Method 208F, page 95 in Standard Methods.	Standard Methods for the Examination of Water and Wastewater. 14th ed., 1975. APHA, Washington, DC 20036.
	This test is used as a Control Check on the proper functioning of treatment processes. Usually, samples are drawn from the raw waste influent and from effluents of the primary and secondary processes. By comparing the quantity of settleable solids among these samples, the effectiveness of removing solids (and turbidity) can be determined. Although the test is not quantitative, it is very useful as a process control test to indicate the volume of sludge which must be withdrawn from a particular process.	Richmond, M.S., et.al. Simplified Laboratory Procedures for Wastewater Examination. Pub. No. 18, 1968. WPCF, Washington, DC 20016.
	This test is listed in the Federal Register "Guidelines Establishing Test Procedures for the Analysis of Pollutants." The only reference cited for the procedure is Standard Methods, 14th ed., page 95.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.2b.	Using an Imhoff cone brush, clean cones with soap or detergent water. Rinse with tap water, shake and invert to drain dry. A few droplets of water may remain on the walls of the cone, especially if plastic cones are used. This is permissible. If stronger cleaning measures are required, rinse cone with dilute acid or chromic acid cleaning mixture, rinse with tap water and drain. (Chromic acid cleaning mixture contains sulfuric acid and should be used carefully. Sulfuric acid causes severe burns.)	Handbook for Analytical Quality Control in Water and Wastewater Laboratories. 1972. U.S. EPA, NERC, Cincinnati, OH 45268
D.2a.	It is always good practice to rinse equipment with tap water as soon as possible after use to facilitate cleaning.	Ibid.
D.3a.	Use cleaning procedure described above in A.2b.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.3a.	COLLECTION OF SAMPLES FOR THIS TEST:	
	You should be familiar with the following information since you record most of it on your laboratory data sheet. You may be responsible for actually collecting the sample; consult your supervisor.	
	Location - Plant control and self-monitoring requirements will be the basis for selecting places to collect samples. Final collection points should be such that samples drawn there are as representative of the entire sample source as possible. Consult your supervisor.	Standard Methods for the Examination of Water and Wastewater. 14th ed., 1975. APHA, Washington, D.C. 20036, p. 38
	Identification - Each collection should be assigned a number or simple identification code. Use this to label samples from that location and to record on the lab data sheet.	
	Type - Collect a grab sample immediately before the test is to be started. Mark type (grab) on sample container and on lab data sheet.	Ibid.
	Time of Collection - Mark date and time on sample container and on lab data sheet.	
	Container - 1000^+ ml volume, capped, resistant (to adsorption of solids) glass or plastic. Clean used containers by rinsing with dilute hydrochloric acid solution, with tap water (three rinses) and with distilled water (three rinses). Shake out excess water.	Ibid, p. 91
	Collection - Rinse container two or three times with sample, then collect about 1000 ml of sample. Exclude very large solids like leaves, sticks, fish, lumps of fecal matter, etc. Put cap on container.	
	Signature - Sample Collector should sign name on container or label so this information can be recorded on the lab data sheet.	
	Storage- It is not practical to preserve and store these samples. Analyze promptly to minimize chemical and/or physical changes.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
B.2b.	Entrained air interferes with settling.	
B.5a.	Vibrations interfere with settling.	
B.5b.	Sunlight can cause heat currents in the sample which interfere with settling.	
C.1c.	It is possible to have a final volume of settled matter greater than the 40 ml (largest) volume marking on the cone. If this happens: 1. Use a grease or wax pencil and put a mark on the outside of the cone at the level of the surface of the settled matter. 2. Discard the sample from the cone. 3. Using a 100 ml graduated cylinder and tap water, fill the cone up to the mark you made on the cone. Keep a count of the volumes of water you add. 4. The total ml of water added to reach the mark you made on the cone are the ml of settled matter you should report as the test result.	

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A.4a. C.2a.	All laboratory records should be kept in a permanently bound book. This is especially important as documentation for any future questions about data required by regulatory agencies.	Handbook for Analytical Quality Control in Water and Wastewater Laboratories. 1972. U.S. EPA, NERC, Cincinnati, OH 45268
A.4c.	Attached as the next page is a typical laboratory data sheet for recording information about this analysis.	
C.4a.	Depending on your organizational set-up, it may be your responsibility to enter the result on the plant operation record, state report form, NPDES report form, etc.. Check with your supervisor.	

Typical Laboratory Data Sheet

for

SETTLEABLE SOLIDS, ml/liter
(Imhoff Settling Cone)

Name of Plant ______________________

STEP	SETTLEABLE SOLIDS	SAMPLE	SAMPLE	SAMPLE
A.4.	Identification			IN #1
A.4.	Type (Grab, etc.)			GRAB
A.4.	Date & Time Collected			9/23/74 9:55
A.4.	Sample Collector			John Sampler
A.5.	Cone Identification			#1
B.1.	Date & Time Analysis began			9/23/74 10:00
C.2.	Final Volume of Settleable Solids, ml/liter			26 ml/liter
C.3.	Analyst			Tom Analyst

A PROTOTYPE FOR DEVELOPMENT OF ROUTINE OPERATIONAL PROCEDURES

for the

REPORTING OF SELF - MONITORING DATA

as applied in

WASTEWATER TREATMENT FACILITIES
and in the
MONITORING OF EFFLUENT WASTEWATERS

Developed by the

National Training and Operational Technology Center
Municipal Operations and Training Division
Office of Water Program Operations
U.S. Environmental Protection Agency

This Procedure was developed by:

NAME Charles E. Sponagle

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POSITION Sanitary Engineer-Instructor

EDUCATION AND TECHNICAL BACKGROUND

B.C.E. - Manhattan College, 1943

M.S. in C.E. - University of Minnesota, 1948

Professional Registration: State of New York

With Federal Water Pollution Control Program since 1948, with various assignments at Program Headquarters, Regional Offices, and Field Stations, including positions as

Staff Engineer, then Chief, Water Quality Section
Denver Regional Office

Staff Engineer, then Regional Construction Grants Program Director, Denver Regional Office

Regional Construction Grants Program Director, Cincinnati Regional Office

Director, Colorado River Basin Water Quality Control Project, Denver Colorado

Industrial Wastes Consultant, Technical Advisory and Investigations Branch, Cincinnati, Ohio

Participation in and Direction of numerous in-plant industrial waste surveys and stream studies in New York, Colorado, New Mexico, Maine, Utah

With National Training Center, September 1969 to date.

EFFLUENT MONITORING PROCEDURE: Reporting of Self-Monitoring Data

1. Objective: To enable the student to complete the NPDES Discharge Monitoring Report, EPA Form T-40(4-74), or EPA Form 3320-1(10-72).

2. Description of Procedure:

Self-monitoring data obtained by a permit holder under the terms of his permit must be reported to the regulatory agency periodically, using the proper NPDES reporting form. The manner in which such data should be reported on EPA Form T-40 is illustrated in this procedure. Additional information required to complete the form is also indicated.

Assumed conditions used to illustrate completion of the form are:

1. Reporting of data on a monthly basis is required.
2. Self-monitoring data developed over a period of one month is as shown in Table I, Page 5
3. Effluent limitations specified in the permit are as shown on Table II, Page 6
4. Monitoring requirements specified in the permit are as shown in Table II, Page 6
5. All required data has been obtained in accordance with permit requirements.

TABLE I
SELF - MONITORING DATA
September 1974

	SEWAGE FLOW	RAW INFLUENT		FINAL EFFLUENT			
Date	Treated gpd	BOD_5 mg/l	T.S.S. mg/l	BOD_5 mg/l	T.S.S. mg/l	Fecal Coliform N/100 ml	pH
1	720,100						7.4
2	609,000						7.5
3	326,900	170	171	16	12	350	7.6
4	367,500						7.4
5	323,900						7.5
6	458,500	160	168	15	16	540	7.7
7	571,000						5.4
8	508,600						7.6
9	146,000	200	200	20	25	180	7.9
10	253,000						7.2
11	406,800						7.1
12	519,200	190	198	20	25	170	7.6
13	328,600						7.5
14	413,100						7.6
15	699,000						8.0
16	708,900	150	180	35	60	220	8.0
17	806,700						9.2
18	714,800						8.0
19	169,100						9.1
20	272,900	170	170	19	19	240	7.5
21	713,200						7.8
22	671,900						7.0
23	761,800	150	186	20	23	110	7.4
24	642,900						7.5
25	314,900						7.4
26	291,600	190	195	20	20	130	7.5
27	240,700						7.4
28	478,900						7.4
29	525,600	190	195	25	25	280	7.6
30	670,100						7.8
Total	14,635,200						
Average	487,800						

EFFLUENT MONITORING PROCEDURE: Reporting of Self-Monitoring Data

TABLE II

EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS

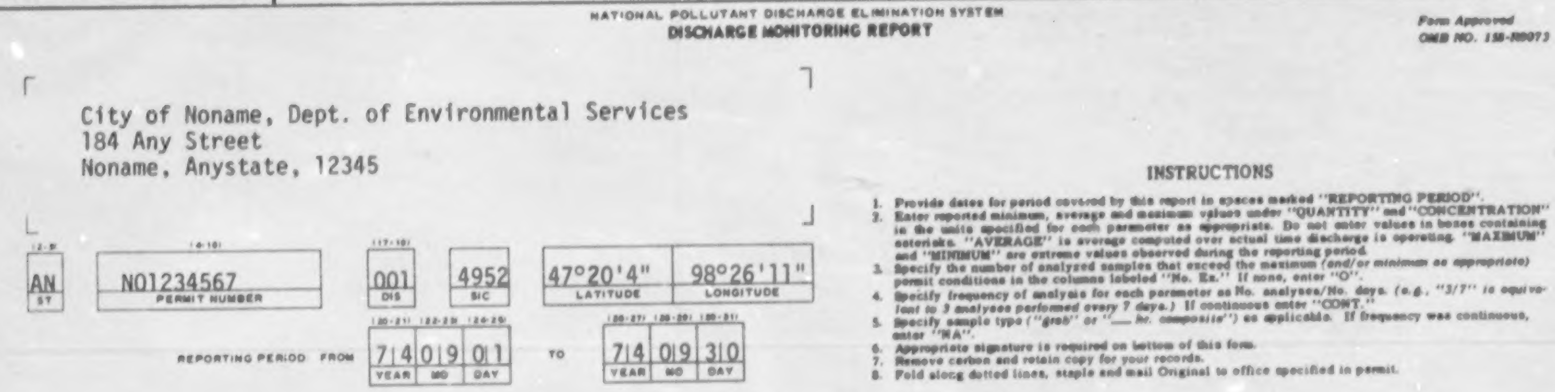
	DISCHARGE LIMITATIONS				MINIMUM MONITORING REQUIREMENTS	
	Concentration in mg/l		kg/day (lbs/day)			
EFFLUENT CHARACTERISTICS	Monthly Average	Weekly Average	Monthly Average	Weekly Average	Measurement Frequency	Sample Type
Biochemical Oxygen Demand (5-day)	*30	45	70 (150)		twice weekly	24 hr. composite
Suspended Solids	*30	45	80 (180)		twice weekly	24 hr. composite
pH - standard units	6.0-9.0	(not to be averaged)			twice weekly	grab
Fecal Coliform - organisms/100 ml	200	400	---	---	twice weekly	grab
Flow - mgd	---	---	---	---	daily	recording

* The arithmetic mean of the values for effluent samples measuring biochemical oxygen demand (5-day) and suspended solids collected in a period of 30 consecutive days shall not exceed 15 percent of the arithmetic mean of the values for influent samples collected at approximately the same times during the same period (85 percent removal--minimum).

* Whichever is the more stringent.

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Description of EPA Forms			I.A (P. 27)
B. Identification of Permit Holder and Discharge	1. Enter Name and Address of Permit Holder	1a. In space provided at left of Instructions 1b. May already be entered by permit-issuing authority.	
	2. Enter State in block labelled "ST"	2a. Use standard two-letter postal code 2b. See notes 1a and 1b above.	
	3. Enter Permit number in block for same.	3a. See notes 1a and 1b above.	
	4. Enter discharge number in "Dis" block.	4a. As identified in permit (001, 002, etc.) 4b. See notes 1a and 1b above.	
	5. Enter Discharge code	5a. For municipal wastewater discharges, the number is 4952. 5b. See notes 1a and 1b above.	
	6. Enter Latitude and Longitude of discharge.	6a. If known. 6b. See notes 1a and 1b above.	
	7. Enter reporting period in appropraite blocks.	7a. In this procedure the 30-day period for the month of September is used. 7b. Will be specified in permit. 7c. See notes 1a and 1b above.	

This portion of the report form, completed in accordance with assumed permit conditions, and the data of Table I, is shown in Fig. 1:



NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM
DISCHARGE MONITORING REPORT

Form Approved
OMB NO. 158-R0073

City of Noname, Dept. of Environmental Services
184 Any Street
Noname, Anystate, 12345

ST: AN
PERMIT NUMBER: N01234567
DIS: 001
SIC: 4952
LATITUDE: 47°20'4"
LONGITUDE: 98°26'11"

REPORTING PERIOD FROM YEAR 74 MO 09 DAY 01 TO YEAR 74 MO 09 DAY 30

INSTRUCTIONS

1. Provide dates for period covered by this report in spaces marked "REPORTING PERIOD".
2. Enter reported minimum, average and maximum values under "QUANTITY" and "CONCENTRATION" in the units specified for each parameter as appropriate. Do not enter values in boxes containing asterisks. "AVERAGE" is average computed over actual time discharge is operating. "MAXIMUM" and "MINIMUM" are extreme values observed during the reporting period.
3. Specify the number of analyzed samples that exceed the maximum *(and/or minimum as appropriate)* permit conditions in the columns labeled "No. Ex." If none, enter "O".
4. Specify frequency of analysis for each parameter as No. analyses/No. days. *(e.g., "3/7" is equivalent to 3 analyses performed every 7 days.)* If continuous enter "CONT."
5. Specify sample type ("grab" or "— hr. composite") as applicable. If frequency was continuous, enter "NA".
6. Appropriate signature is required on bottom of this form.
7. Remove carbon and retain copy for your records.
8. Fold along dotted lines, staple and mail Original to office specified in permit.

Fig. 1

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Flow Data.			
1. "Quantity" section.	1. Enter minimum and maximum flows during the reporting period in the corresponding spaces on the "reported" line.	1a. Minimum flow of 0.15 MGD on Sept. 9. (Table I) 1b. Maximum flow of 0.81 MGD on Sept. 17. (Table I)	IX.C.1.1 (p. 35)
	2. Enter Average flow during reporting period in corresponding space on "reported" line.	2a. Add the flows reported during the reporting period. Divide this total by the number of flows reported. 2b. From Table I, Average = $\frac{14,635,200}{30}$ = 0.49 MGD	
	3. Enter on "Permit Condition" line the average and maximum daily flows specified in the permit.	3a. If unspecified in Permit, place a dash in the appropriate space or spaces. 3b. May already have been entered by permit-issuing authority.	IX.C.1.3.3a (p. 35)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Flow Data (cont.)	4. On the "Reported" line, in the "No. Ex" space, enter the number of times during the reporting period that the maximum daily flow specified in the permit was exceeded	4a. If none, enter "0". 4b. If a maximum daily flow is not specified in the permit, place a dash in this space.	
2. "Concentration" section	1. Place dashes in the "Units"space and in the "No. Ex" spaces on the "Reported" line.	1a. May already be entered by the Permit-issuing authority.	
3. "Frequency of Analysis" Column	1. On the "Reported" line enter the frequency with which flows were measured during the reporting period.	1a. Daily, Weekly, Continuous (Cont.), etc.	
	2. On the "Permit Condition" line enter the frequency of flow measurement as specified in the permit.	2a. May already be entered by Permit-issuing authority.	
4. "Sample Type" Column	1. Enter Dashes on both lines.		

This portion of the report form, completed in accordance with assumed permit conditions, and the data of Table I, is shown in Fig. 2:

		(3 card only) QUANTITY					(4 card only) CONCENTRATION						
PARAMETER		MINIMUM	AVERAGE	MAXIMUM	UNITS	NO. EX	MINIMUM	AVERAGE	MAXIMUM	UNITS	NO. EX	FREQUENCY OF ANALYSIS	SAMPLE TYPE
FLOW	REPORTED	0.15	0.49	0.81	MGD	-	******	******	******	-	-	Cont.	-
	PERMIT CONDITION	******	-	-		■	******	******	******		■	Cont.	-

Fig. 2

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. pH Data			
1. "Quantity" section	1. On the "Reported" line enter the minimum and maximum pH values occurring during the reporting period.	1a. Although the permit requires that pH be determined twice weekly, a pH was run each day during the month. The report form must be prepared on the basis of all 30 results. 1b. Minimum pH 5.4 on Sept. 7 (Table I) 1c. Maximum pH 9.2 on Sept. 17. (Table I)	
	2. On the "Permit Condition" line enter the minimum and maximum pH values specified in the permit.	2a. Permit requires pH to be between 6.0 and 9.0 at all times (Table II) 2b. May already be entered on the form by the permit-issuing authority.	
	3. On the "Reported" line, in the "No. Ex" space, enter the total number of times that the pH exceeded the maximum allowed by the permit, and was less than the minimum allowed by the permit.	3a. The maximum permit requirement of 9.0 was exceeded twice - once on Sept. 17, and again on Sept. 19. (Table I) 3b. The pH was less than the minimum permit requirement of 6.0 on Sept. 7. (Table I)	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
D. pH Data (cont.)		3c. Permit requirements were violated a total of 3 times during the reporting period. A "3" should be entered on the Form.	
		3d. If the pH had not exceeded the permit limits, a "0" would be entered.	
2. "Concentration" section	1. Place dashes in the "Units" space and in the "No. Ex" space on the "Reported" line.	1a. May already be entered by Permit-issuing authority.	
3. "Frequency of Analysis" column.	1. Enter frequency of analysis during the reporting period on the "Reported" line.	1a. The actual frequency of analysis is reported. 1b. pH was run each day. The frequency of analysis is reported as 7/7, which indicates that 7 analyses were performed every seven days.	
	2. Enter required frequency of analysis as specified in permit on the "Permit Condition" line.	2a. The permit requires that pH be run twice weekly. (Table II) A 2/7 is entered on this line, which indicates that 2 analyses were to be performed every 7 days. 2b. May already be entered by the Permit-issuing authority.	
4. "Sample Type" column.	1. On the "Reported" line indicate the type of sample on which the analysis was performed.	1a. A grab sample is assumed here. "Grab" is entered.	
	2. On the "Permit Condition" line enter the type of sample specified by the permit.	2a. A grab sample is specified. (Table II) Enter "Grab". 2b. May already be entered by the Permit-issuing authority.	

D. pH Data (cont.)

This portion of the report form, completed in accordance with assumed permit conditions, and the data of Table I, is shown in Fig. 3 below:

(32-37) PARAMETER		(3 card only) QUANTITY (38-45)	(46-53)	(54-61)		(62-63)	(4 card only) CONCENTRATION (38-45)	(46-53)	(54-61)		(62-63)	(64-68)	(69-70)
		MINIMUM	AVERAGE	MAXIMUM	UNITS	NO. EX	MINIMUM	AVERAGE	MAXIMUM	UNITS	NO. EX	FREQUENCY OF ANALYSIS	SAMPLE TYPE
PH	REPORTED	5.4	******	9.2	STANDARD UNITS	3	******	******	******	-	-	7/7	Grab
	PERMIT CONDITION	6.0	******	9.0		■	******	******	******		■	2/7	Grab

Fig. 3

E. BOD$_5$ Data, Final Effluent.			
1. Computation of Quantities	1. For each reported analytical result, calculate the quantity of BOD$_5$ discharged in Kg/day.		II.E.1 (p. 30)
2. "Quantity" section	1. On the "Reported" line enter the minimum, average, and maximum quantities discharged over the reporting period, in the appropriate spaces.	1a. Minimum - 11.0 Kg/day 1b. Average - 37.5 Kg/day 1c. Maximum - 94 Kg/day	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. BOD_5 Data, Final Effluent.(cont.)	2. On the "Permit Condition" line enter the average and maximum quantities permitted to be discharged.	2a. Average BOD_5 discharge over a 30-consecutive-day period is specified as 70 Kg/day. (Table II) 2b. A Maximum daily discharge limitation is not indicated in the permit. (Table II) A dash is therefore placed in the "maximum" space. 2c. May already be entered by the Permit-issuing authority.	
	3. In the "No. Ex" column, on the "Reported" line, enter the number of times that the maximum daily discharge of BOD_5 (Kg/day) allowed by the permit has been exceeded during the reporting period.	3a. If none, enter a "0". 3b. Since no maximum daily discharge limitation is specified in the permit (Table II), a dash is placed in this space.	
3. "Concentration" section.	1. On the "Reported" line enter the minimum, average, and maximum BOD_5 concentrations observed during the reporting period.	1a. Minimum - 15 mg/l 1b. Average - 21 mq/l 1c. Maximum - 35 mg/l	II.E.1 (p. 30)
	2. On the "Permit Condition" line enter the average and maximum concentrations specified in the permit.	2a. Average BOD_5 concentration over a 30-consecutive-day period is specified as 30 mg/l. (Table II) 2b. A maximum concentration is not indicated in the permit conditions (Table II). Therefore place a dash in this space. 2c. May already be entered by the Permit-issuing authority.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
E. BOD_5 Data, Final Effluent.(cont.)	3. In the "No. Ex" column, the "Reported" line, enter the number of times that the maximum concentration allowed by the permit has been exceeded during the reporting period.	3a. If none, enter a "0". 3b. Since no maximum concentration is specified in the permit (Table II) enter a dash in this space.	
4. "Frequency of Analysis" column	1. On the "Reported" line enter frequency of analysis during the reporting period.	1a. Enter 2/7, indicating that the analysis was performed twice weekly (Table I).	
	2. On the "Permit Condition" line enter required frequency of analysis as specified in permit.	2a. Permit requires analysis twice weekly (Table II). Enter 2/7. 2b. May already be entered by the Permit-issuing authority.	
5. "Sample Type" column	1. On the "Reported" line enter the type of sample on which the analysis was performed.	1a. Enter "24-Hr. comp." since it is assumed in this procedure that the data has been obtained in accordance with permit requirements.	
	2. On the "Permit Condition" line enter the type of sample specified in the permit.	2a. Enter "24-Hr. comp." (Table II). 2b. May already be entered by the Permit-issuing authority.	

This portion of the Report Form, completed in accordance with assumed permit conditions, and the data of Table I, is shown in Fig. 4:

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES

PARAMETER (32-37)		QUANTITY (3 card only) MINIMUM (38-45)	QUANTITY AVERAGE (46-53)	QUANTITY MAXIMUM (54-61)	UNITS	NO. EX (62-63)	CONCENTRATION (4 card only) MINIMUM (38-45)	CONCENTRATION AVERAGE (46-53)	CONCENTRATION MAXIMUM (54-61)	UNITS	NO. EX (62-63)	FREQUENCY OF ANALYSIS (64-68)	SAMPLE TYPE (69-70)
BOD_5	REPORTED	11.0	37.5	94	KG/DAY	-	15	21	35	MG/L	-	2/7	24-Hr.Comp
	PERMIT CONDITION	******	70	-		■	******	30	-		■	2/7	24-Hr.Comp

Fig. 4

F. Percent Removal BOD_5 1. Computation	1. Calculate the percent BOD_5 removal for each pair of influent and effluent analyses made during the reporting period.		II.F.1 (p. 31)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
F. Percent Removal BOD_5 (cont.)			
2. "Quantity" section	1. On the "Reported" line enter the minimum, average, and maximum percent removals in the appropriate spaces.	1a. Minimum - 77% 1b. Average - 87.9% 1c. Maximum - 91%	
	2. On the "Permit Condition" line enter the minimum and average removals required by the permit.	2a. There is no minimum removal requirement in the assumed permit conditions (Table II). Enter a dash in this space. 2b. Average removal required over a 30-consecutive-day period is 85%. (Table II). 2c. May already be entered by the Permit-issuing authority.	
	3. In the "No. Ex" column, on the "Reported" line, enter the number of times that the minimum percent removal required in the permit was not obtained.	3a. There is no minimum removal requirement in the assumed permit conditions (Table II). Enter a dash in this space.	
3. "Concentration" section	1. Enter dashes in the "Units" space and in the "No. Ex" space on the "Reported" line.	1a. May already be entered by the Permit-issuing authority.	

This portion of the Report Form, completed in accordance with assumed permit conditions, and the data of Table I, is shown in Fig. 5:

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES

PARAMETER		QUANTITY (3 card only) MINIMUM	QUANTITY AVERAGE	QUANTITY MAXIMUM	UNITS	NO. EX	CONCENTRATION (4 card only) MINIMUM	CONCENTRATION AVERAGE	CONCENTRATION MAXIMUM	UNITS	NO. EX	FREQUENCY OF ANALYSIS	SAMPLE TYPE
PERCENT REMOVAL BOD$_5$	REPORTED	77	87.9	91	%	-	******	******	******	-	-	******	******
	PERMIT CONDITION	-	85	******		■	******	******	******		■	******	******

Fig. 5

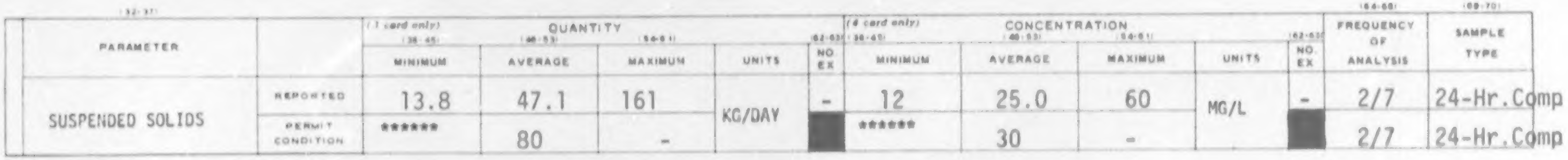
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
G. Suspended Solids, Final Effluent			
1. Computation of Quantities	1. For each reported analytical result, calculate the quantity of suspended solids discharged in Kg/day.		II.G.1 (p. 32)
2. "Quantity" section	1. On the "Reported" line enter the minimum, average, and maximum quantities discharged over the reporting period, in the corresponding spaces.	1a. Minimum - 13.8 kg/day 1b. Average - 47.1 kg/day 1c. Maximum - 161 kg/day	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
G. Suspended Solids, Final Effluent (cont.)	2. On the "Permit Condition" line enter the average and maximum quantities permitted to be discharged.	2a. Average suspended solids discharge over a 30-consecutive-day period is specified as 80 kg/day (Table II) 2b. A maximum daily discharge limitation is not specified in the permit (Table II). A dash is therefore placed in the "maximum" space. 2c. May already be entered by the Permit-issuing authority.	
	3. In the "No. Ex" column, on the "Reported" line, enter the number of times that the maximum daily discharge of suspended solids (kg/day) has been exceeded during the reporting period.	3a. If none, enter a "0". 3b. Since no maximum daily discharge limitation is indicated in the permit (Table II), a dash is placed in this space.	
3. "Concentration" section.	1. On the "Reported" line enter the minimum, average, and maximum suspended solids concentration observed during the reporting period, in the corresponding spaces.	1a. Minimum - 12 mg/l 1b. Average - 25.0 mg/l 1c. Maximum - 60 mg/l	
	2. On the "Permit Condition" line enter the average and maximum concentrations specified in the permit, in the appropriate spaces.	2a. Average suspended solids concentration over a 30-consecutive-day period is specified as 30 mg/l (Table II).	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
G. Suspended Solids, Final Effluent. (cont.)		2b. Since no maximum concentration is specified in the permit (Table II), enter a dash in this space.	
		2c. May already be entered by the Permit-issuing authority.	
	3. In the "No. Ex" column, on the "Reported" line, enter the number of times during the reporting period that the maximum concentration allowed by the permit has been exceeded.	3a. If none, enter a "0". 3b. Since no maximum concentration is specified in the permit (Table II), enter a dash in this space.	
4. "Frequency of Analysis" column	1. On the "Reported" line enter frequency of analysis during the reporting period.	1a. Enter 2/7, indicating that the analysis was performed twice weekly (Table I).	
	2. On the "Permit Condition" line enter required frequency of analysis, as specified in permit.	2a. Permit requires analysis twice weekly (Table II). Enter 2/7. 2b. May already be entered by Permit-issuing authority.	
5. "Sample Type" column.	1. On the "Reported" line enter the type of sample on which the analysis was performed.	1a. Enter "24-Hr. comp." since it is assumed in this procedure that the data has been obtained in accordance with permit requirements.	
	2. On the "Permit Condition" line enter the type of sample specified in the permit.	2a. Enter "24-Hr. comp." (Table II). 2b. May already be entered by the Permit-issuing authority.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
G. Suspended Solids, Final Effluent. (cont.)			

This portion of the Report Form, completed in accordance with assumed permit conditions, and the data of Table I, is shown in Fig. 6 below:



PARAMETER (32-37)		QUANTITY (1 card only) MINIMUM (38-45)	QUANTITY AVERAGE (46-53)	QUANTITY MAXIMUM (54-61)	UNITS	NO EX (62-63)	CONCENTRATION (4 card only) MINIMUM (38-45)	CONCENTRATION AVERAGE (46-53)	CONCENTRATION MAXIMUM (54-61)	UNITS	NO. EX (62-63)	FREQUENCY OF ANALYSIS (64-68)	SAMPLE TYPE (69-70)
SUSPENDED SOLIDS	REPORTED	13.8	47.1	161	KG/DAY	-	12	25.0	60	MG/L	-	2/7	24-Hr.Comp
	PERMIT CONDITION	******	80	-		■	******	30	-		■	2/7	24-Hr.Comp

Fig. 6

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
H. Percent Removal Suspended Solids.			
1. Computation.	1. Calculate the percent removal of suspended solids for each pair of influent and effluent analyses made during the reporting period.		II.H.1 (p.33)
2. "Quantity" section	1. On the "Reported" line enter the minimum, average, and maximum percent removals in the corresponding spaces.	1a. Minimum - 66.6% 1b. Average - 86.5% 1c. Maximum - 93.0%	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
H. Percent Removal Suspended Solids.(cont.)	2. On the "Permit Condition" line enter the minimun and average removals required by the permit.	2a. There is no minimum removal requirement in the assumed permit conditions (Table II). Enter a dash in this space. 2b. Average removal required over a 30-consecutive-day period is 85% (Table II). 2c. May already be entered by the permit-issuing authority.	
	3. In the "No. Ex" column, on the "Reported" line, enter the number of times that the minimum percent removal required in the permit was not obtained.	3a. There is no minimum removal requirement in the assumed permit conditions (Table II). Enter a dash in this space. 3b. If none, enter a "0".	
3. "Concentration" section.	1. Enter dashes in the "Units" space and in the "No. Ex" space on the "Reported" line.	1a. May already be entered by the Permit-issuing authority.	

This portion of the form, completed in accordance with assumed permit conditions, and the data of Table I, is shown in Fig. 7 below:

(32-37) PARAMETER		(3 card only) QUANTITY					(4 card only) CONCENTRATION					(64-68) FREQUENCY OF ANALYSIS	(69-71) SAMPLE TYPE
		(38-45) MINIMUM	(46-53) AVERAGE	(54-61) MAXIMUM	UNITS	(62-63) NO EX	(38-45) MINIMUM	(46-53) AVERAGE	(54-61) MAXIMUM	UNITS	(62-63) NO EX		
PERCENT REMOVAL SUSPENDED SOLIDS	REPORTED	66.6	86.5	93.0	%	-	******	******	******	-	-	******	******
	PERMIT CONDITION	-	85	******		■	******	******	******		■	******	******

Fig. 7

567

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
I. Fecal Coliform			
1. Computation	1. Calculate the geometric mean for the fecal coliform data obtained during the reporting period.	1a. See also the effluent monitoring procedure Calculation of the Geometric Mean of Coliform Counts by the Use of Logarithms.	II.I.1 (p. 34)
2. "Quantity" section	1. Place dashes in the "Units" space and in the "No. Ex" space on the "Reported" line.	1a. May already be entered by the Permit-issuing authority.	
3. "Concentration" section.	1. Enter the minimum and maximum reported results in the corresponding spaces on the "Reported" line.	1a. Minimum - 110 organisms/100 ml 1b. Maximum - 540 organisms/100 ml	
	2. Enter the geometric mean in the "average" space on the "Reported" line.	2a. Geometric Mean - 220 organisms/100 ml	
	3. On the "Permit Condition" line enter the geometric mean and maximum count specified in the permit.	3a. The geometric mean of samples analyzed over a 30-consecutive-day period is not to exceed 200 organisms/100 ml. (Table II) Enter "200" in "average" space. 3b. There is no maximum value specified in the permit (Table II). Enter a dash in the "maximum" space.	

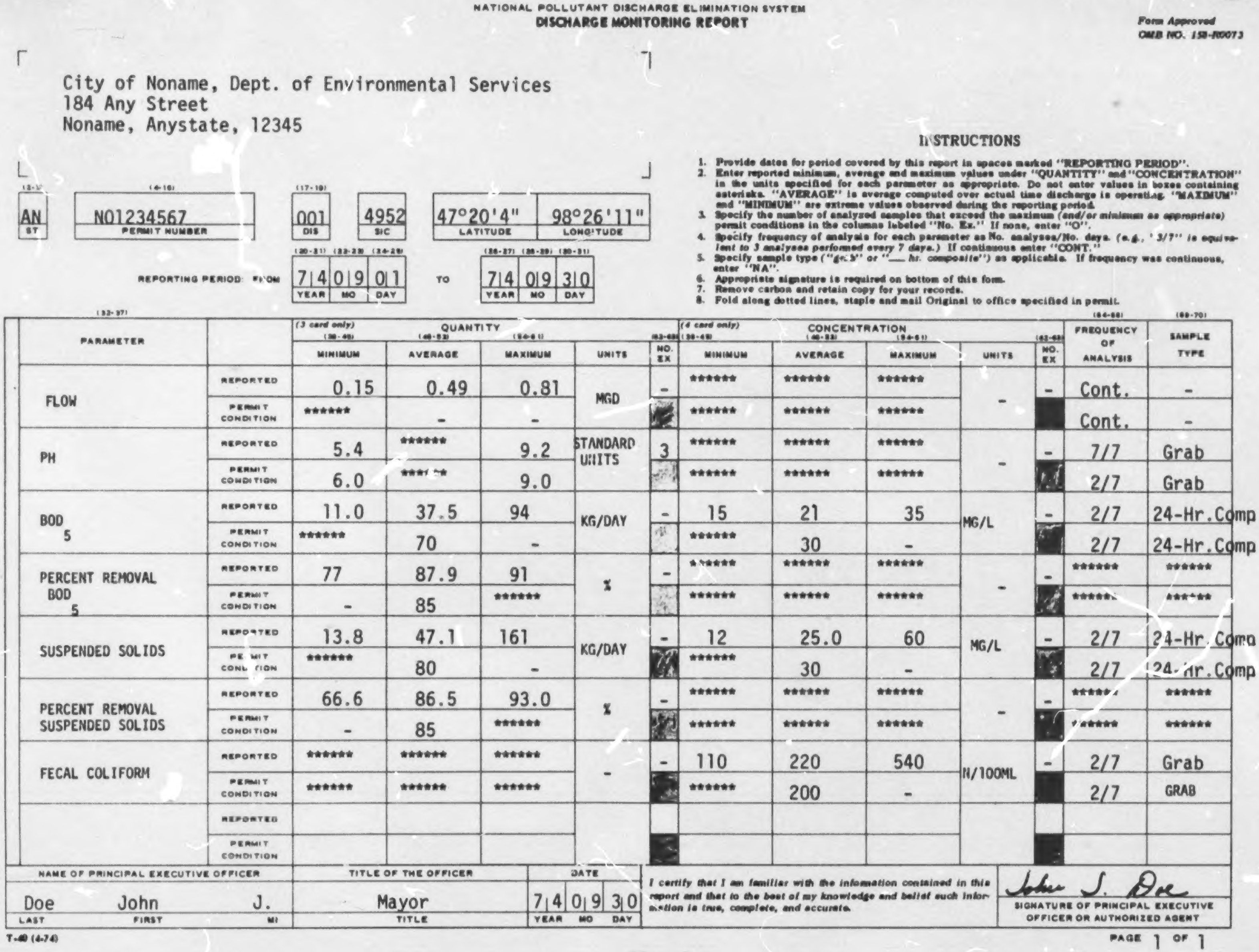
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
I. Fecal Coliform. (cont.)	4. In the "No. Ex" column, on the "Reported" line, enter the number of times during the reporting period that the maximum count allowed in the permit has been exceeded.	4a. If none, enter a "0". 4b. Since no maximum count is specified in the permit (Table II) enter a dash in this space.	
4. "Frequency of Analysis" column	1. Enter frequency of analysis during the reporting period on the "Reported" line.	1a. Enter 2/7, indicating that the analysis was performed twice weekly (Table I).	
	2. Enter required frequency of analysis, as specified in permit, on the "Permit Condition" line.	2a. Permit requires analysis twice weekly (Table II). Enter 2/7.	
5. "Sample Type" column.	1. Enter type of sample on which the analysis was performed.	1a. Enter "Grab", since it is assumed in this procedure that the sample has been obtained in accordance with permit requirements.	

This portion of the form, completed in accordance with assumed permit conditions, and the data of Table I, is shown in Fig. 8 below:

(32-37) PARAMETER		(3 card only) QUANTITY					(4 card only) CONCENTRATION					(64-68) FREQUENCY OF ANALYSIS	(69-70) SAMPLE TYPE
		(38-45) MINIMUM	(46-53) AVERAGE	(54-61) MAXIMUM	UNITS	(62-63) NO. EX	(38-45) MINIMUM	(46-53) AVERAGE	(54-61) MAXIMUM	UNITS	(62-63) NO. EX		
FECAL COLIFORM	REPORTED	******	******	******	-	-	110	220	540	N/100ML	-	2/7	Grab
	PERMIT CONDITION	******	******	******		■	******	200	-		■	2/7	GRAB

Fig. 8

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
J. Signature	1. The completed form must be signed by the ranking elected official of the municipality, or other duly authorized municipal employee.		
	2. Complete the four spaces provided at the bottom of the form.	2a. Name, title and signature of ranking elected official or duly authorized employee, and date of completion.	
	3. Forward completed form to permit-issuing authority in accordance with reporting instructions specified in permit.	3a. The entire form, completed in accordance with the permit conditions assumed for the purpose of this procedure, is shown in Fig. 9.	



NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM
DISCHARGE MONITORING REPORT

Form Approved
OMB NO. 158-R0073

City of Noname, Dept. of Environmental Services
184 Any Street
Noname, Anystate, 12345

(2-3) ST	(4-10) PERMIT NUMBER	(17-19) DIS	SIC	LATITUDE	LONGITUDE
AN	N01234567	001	4952	47°20'4"	98°26'11"

REPORTING PERIOD: FROM 74 (YEAR) 09 (MO) 01 (DAY) TO 74 (YEAR) 09 (MO) 30 (DAY)

INSTRUCTIONS

1. Provide dates for period covered by this report in spaces marked "REPORTING PERIOD".
2. Enter reported minimum, average and maximum values under "QUANTITY" and "CONCENTRATION" in the units specified for each parameter as appropriate. Do not enter values in boxes containing asterisks. "AVERAGE" is average computed over actual time discharge is operating. "MAXIMUM" and "MINIMUM" are extreme values observed during the reporting period.
3. Specify the number of analyzed samples that exceed the maximum *(and/or minimum as appropriate)* permit conditions in the columns labeled "No. Ex." If none, enter "O".
4. Specify frequency of analysis for each parameter as No. analyses/No. days. *(e.g., "3/7" is equivalent to 3 analyses performed every 7 days.)* If continuous enter "CONT."
5. Specify sample type *("grab" or "— hr. composite")* as applicable. If frequency was continuous, enter "NA".
6. Appropriate signature is required on bottom of this form.
7. Remove carbon and retain copy for your records.
8. Fold along dotted lines, staple and mail Original to office specified in permit.

(32-37) PARAMETER		(3 card only) QUANTITY (38-45)	(46-53)	(54-61)		(62-63)	(4 card only) CONCENTRATION (38-45)	(46-53)	(54-61)		(62-63)	(64-68) FREQUENCY OF ANALYSIS	(69-70) SAMPLE TYPE
		MINIMUM	AVERAGE	MAXIMUM	UNITS	NO. EX	MINIMUM	AVERAGE	MAXIMUM	UNITS	NO. EX		
FLOW	REPORTED	0.15	0.49	0.81	MGD	-	******	******	******	-	-	Cont.	-
	PERMIT CONDITION	******	-	-			******	******	******			Cont.	-
PH	REPORTED	5.4	******	9.2	STANDARD UNITS	3	******	******	******	-	-	7/7	Grab
	PERMIT CONDITION	6.0	******	9.0			******	******	******			2/7	Grab
BOD $_5$	REPORTED	11.0	37.5	94	KG/DAY	-	15	21	35	MG/L	-	2/7	24-Hr.Comp
	PERMIT CONDITION	******	70	-			******	30	-			2/7	24-Hr.Comp
PERCENT REMOVAL BOD $_5$	REPORTED	77	87.9	91	%	-	******	******	******	-	-	******	******
	PERMIT CONDITION	-	85	******			******	******	******			******	******
SUSPENDED SOLIDS	REPORTED	13.8	47.1	161	KG/DAY	-	12	25.0	60	MG/L	-	2/7	24-Hr.Comp
	PERMIT CONDITION	******	80	-			******	30	-			2/7	24-Hr.Comp
PERCENT REMOVAL SUSPENDED SOLIDS	REPORTED	66.6	86.5	93.0	%	-	******	******	******	-	-	******	******
	PERMIT CONDITION	-	85	******			******	******	******			******	******
FECAL COLIFORM	REPORTED	******	******	******	-	-	110	220	540	N/100ML	-	2/7	Grab
	PERMIT CONDITION	******	******	******			******	200	-			2/7	GRAB
	REPORTED												
	PERMIT CONDITION												

NAME OF PRINCIPAL EXECUTIVE OFFICER			TITLE OF THE OFFICER	DATE		
Doe	John	J.	Mayor	74	09	30
LAST	FIRST	MI	TITLE	YEAR	MO	DAY

I certify that I am familiar with the information contained in this report and that to the best of my knowledge and belief such information is true, complete, and accurate.

John J. Doe
SIGNATURE OF PRINCIPAL EXECUTIVE OFFICER OR AUTHORIZED AGENT

T-40 (4-74)

PAGE 1 OF 1

Fig. 9

EFFLUENT MONITORING PROCEDURE: REPORTING OF SELF-MONITORING DATA

TRAINING GUIDE

SECTION	TOPIC
*I	Introduction
*II	Educational Concepts - Mathematics
III	Educational Concepts - Science
IV	Educational Concepts - Communication
V	Field & Laboratory Equipment
VI	Field & Laboratory Reagents
VII	Field & Laboratory Analysis
VIII	Safety
*IX	Records & Reports

Training guide materials are presented here under the headings marked. These standardized headings are used throughout this series of procedures.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
A	Holders of discharge permits issued by the U. S. Environmental Protection Agency are to report self-monitoring data either on EPA Form T-40 (Fig. 10), or on EPA Form 3320-1 (Fig. 11). Form T-40 will be used temporarily, and will be furnished by EPA to municipalities for reporting purposes. The T-40 form shown in Fig. 10 consists of the 3320-1 form, which has been **preprinted** for the reporting of data for basic parameters common to all municipal wastewater discharges. As information for each municipality is incorporated into EPA's computer system, form 3320-1 will replace Form T-40. For data reporting, a municipality will then receive from EPA Form 3320-1 on which the effluent parameters specific to that municipality will be computer preprinted. Until that time, however, data for any additional parameters to be reported which are not now included in the preprint on T-40 will be entered by the municipality, using as many additional blank copies of the form as are required. Completion of form T-40, is illustrated in this procedure for the basic parameters, assuming that permit conditions are as indicated in Table II. Reporting of additional parameters would be done in a [illegible]er similar to that illustrated.	

NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM

DISCHARGE MONITORING REPORT

Form Approved
OMB NO. 158-R0073

(2-3)	(4-16)	(17-19)			
ST	PERMIT NUMBER	DIS	SIC	LATITUDE	LONGITUDE

REPORTING PERIOD: FROM (20-21) YEAR (22-23) MO (24-25) DAY TO (26-27) YEAR (28-29) MO (30-31) DAY

INSTRUCTIONS

1. Provide dates for period covered by this report in spaces marked "REPORTING PERIOD".
2. Enter reported minimum, average and maximum values under "QUANTITY" and "CONCENTRATION" in the units specified for each parameter as appropriate. Do not enter values in boxes containing asterisks. "AVERAGE" is average computed over actual time discharge is operating. "MAXIMUM" and "MINIMUM" are extreme values observed during the reporting period.
3. Specify the number of analyzed samples that exceed the maximum *(and/or minimum as appropriate)* permit conditions in the columns labeled "No. Ex." If none, enter "O".
4. Specify frequency of analysis for each parameter as No. analyses/No. days. *(e.g., "3/7" is equivalent to 3 analyses performed every 7 days.)* If continuous enter "CONT."
5. Specify sample type (*"grab"* or *"— hr. composite"*) as applicable. If frequency was continuous, enter "NA".
6. Appropriate signature is required on bottom of this form.
7. Remove carbon and retain copy for your records.
8. Fold along dotted lines, staple and mail Original to office specified in permit.

PARAMETER (32-37)		QUANTITY (3 card only) MINIMUM (38-45)	AVERAGE (46-53)	MAXIMUM (54-61)	UNITS	NO. EX (62-63)	CONCENTRATION (4 card only) MINIMUM (38-45)	AVERAGE (46-53)	MAXIMUM (54-61)	UNITS	NO. EX (62-63)	FREQUENCY OF ANALYSIS (64-68)	SAMPLE TYPE (69-70)
FLOW	REPORTED				MGD		******	******	******				
	PERMIT CONDITION	******				■	******	******	******		■		
PH	REPORTED		******		STANDARD UNITS		******	******	******				
	PERMIT CONDITION		******			■	******	******	******		■		
BOD$_5$	REPORTED				KG/DAY					MG/L			
	PERMIT CONDITION	******				■	******				■		
PERCENT REMOVAL BOD$_5$	REPORTED				%		******	******	******			******	******
	PERMIT CONDITION			******		■	******	******	******		■	******	******
SUSPENDED SOLIDS	REPORTED				KG/DAY					MG/L			
	PERMIT CONDITION	******				■	******				■		
PERCENT REMOVAL SUSPENDED SOLIDS	REPORTED				%		******	******	******			******	******
	PERMIT CONDITION			******		■	******	******	******		■	******	******
FECAL COLIFORM	REPORTED	******	******	******						N/100ML			
	PERMIT CONDITION	******	******	******		■	******				■		GRAB
	REPORTED												
	PERMIT CONDITION					■					■		

NAME OF PRINCIPAL EXECUTIVE OFFICER	TITLE OF THE OFFICER	DATE
LAST FIRST MI	TITLE	YEAR MO DAY

I certify that I am familiar with the information contained in this report and that to the best of my knowledge and belief such information is true, complete, and accurate.

SIGNATURE OF PRINCIPAL EXECUTIVE OFFICER OR AUTHORIZED AGENT

PAGE OF

T-40 (4-74)

Fig. 10

NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM
DISCHARGE MONITORING REPORT

Form Approved
OMB NO. 158-R0073

INSTRUCTIONS

1. Provide dates for period covered by this report in spaces marked "REPORTING PERIOD".
2. Enter reported minimum, average and maximum values under "QUANTITY" and "CONCENTRATION" in the units specified for each parameter as appropriate. Do not enter values in boxes containing asterisks. "AVERAGE" is average computed over actual time discharge is operating. "MAXIMUM" and "MINIMUM" are extreme values observed during the reporting period.
3. Specify the number of analyzed samples that exceed the maximum *(and/or minimum as appropriate)* permit conditions in the columns labeled "No. Ex." If none, enter "O".
4. Specify frequency of analysis for each parameter as No. analyses/No. days. *(e.g., "3/7" is equivalent to 3 analyses performed every 7 days.)* If continuous enter "CONT."
5. Specify sample type (*"grab"* or *"— hr. composite"*) as applicable. If frequency was continuous, enter "NA".
6. Appropriate signature is required on bottom of this form.
7. Remove carbon and retain copy for your records.
8. Fold along dotted lines, staple and mail Original to office specified in permit.

(2-3)	(4-16)	(17-19)			
ST	PERMIT NUMBER	DIS	SIC	LATITUDE	LONGITUDE

REPORTING PERIOD	FROM	(20-21)	(22-23)	(24-25)	TO	(26-27)	(28-29)	(30-31)
		YEAR	MO	DAY		YEAR	MO	DAY

(32-37) PARAMETER		QUANTITY *(3 card only)* (38-45) MINIMUM	(46-53) AVERAGE	(54-61) MAXIMUM	UNITS	(62-63) NO. EX.	CONCENTRATION *(4 card only)* (38-45) MINIMUM	(46-53) AVERAGE	(54-61) MAXIMUM	UNITS	(62-63) NO. EX	(64-68) FREQUENCY OF ANALYSIS	(69-70) SAMPLE TYPE
	REPORTED												
	PERMIT CONDITION					▨					▨		
	REPORTED												
	PERMIT CONDITION					▨					▨		
	REPORTED												
	PERMIT CONDITION					▨					▨		
	REPORTED												
	PERMIT CONDITION					▨					▨		
	REPORTED												
	PERMIT CONDITION					▨					▨		
	REPORTED												
	PERMIT CONDITION					▨					▨		
	REPORTED												
	PERMIT CONDITION					▨					▨		
	REPORTED												
	PERMIT CONDITION					▨					▨		

NAME OF PRINCIPAL EXECUTIVE OFFICER	TITLE OF THE OFFICER	DATE	*I certify that I am familiar with the information contained in this report and that to the best of my knowledge and belief such information is true, complete, and accurate.*	
LAST FIRST MI	TITLE	YEAR MO DAY		SIGNATURE OF PRINCIPAL EXECUTIVE OFFICER OR AUTHORIZED AGENT

EPA Form 3320-1 (10-72)

PAGE OF

Fig. 11

TRAINING GUIDE NOTE

NOTE: In all of the calculations in this section, rules for computation as given by Crumpler and Yoe were followed. Crumpler, T. B. and Yoe, J. H. Chemical Computations and Errors. Wiley and Sons. N.Y. 1940.

E.1 Computation of Quantities of BOD discharged in Final Effluent.

Pertinent reported data from Table I is listed in the first three columns of the Table below. In column 4 the flow has been converted from gpd to MGD. The quantity of BOD_5 is obtained by multiplying the value in column 3 (mg/l) by the value in column 4 (MGD), and then multiplying the result by the factor 3.78.

This is expressed in mathematical form as

Kg/day = MGD x mg/l x 3.78

Example:

On September 3, Kg/day = 0.33 x 16 x 3.78 = 20

Date	Flow gpd	BOD_5 mg/l	Flow MGD	BOD_5 Kg/day
3	326,900	16	0.33	20
6	458,500	15	0.46	26
9	146,000	20	0.146	11.0
12	519,200	20	0.52	39
16	708,900	35	0.71	94
20	272,900	19	0.27	19
23	761,800	20	0.76	57
26	291,600	20	0.29	22
29	525,600	25	0.53	50
Total		190		338

$$\text{Average } BOD_5 = \frac{190}{9} = 21 \text{ mg/l}$$

$$\text{Average } BOD_5 = \frac{338}{9} = 37.5 \text{ Kg/day}$$

REFERENCES/RESOURCES

Crumpler, T. B. and Yoe, J. H. Chemical Computations and Errors. Wiley and Sons. N.Y. 1940.

TRAINING GUIDE NOTE		REFERENCES/RESOURCES

F.1

Computation of percent BOD_5 removals.

Pertinent reported data from Table I is listed in the first three columns of the Table below. The percent BOD_5 removal for each day appears in column 4.

The percent removal is obtained by subtracting the concentration of BOD_5 in the final effluent from that in the plant influent, dividing this difference by the concentration of BOD_5 in the influent, and multiplying the result by 100. This can be expressed in mathematical form as follows:

$$\%BOD_5 \text{ Removal} = \frac{\text{Influent } BOD_5 \text{ (mg/l)} - \text{Effluent } BOD_5 \text{ (mg/l)}}{\text{Influent } BOD_5 \text{ (mg/l)}} \times 100$$

Example:

$$\text{On September 3, \% BOD Removal} = \frac{170 - 16}{170} \times 100 = 91\%$$

Date	BOD_5-mg/l Inf.	BOD_5-mg/l Eff.	% Removal
3	170	16	91
6	160	15	91
9	200	20	90
12	190	20	89
16	150	35	77
20	170	19	89
23	150	20	87
26	190	20	89
29	190	25	87
Total	1,570	190	
Average	174	21	

$$\text{Average } BOD_5 \text{ Removal} = \frac{174-21}{174} \times 100 = 87.9\%$$

G.1 Computation of Quantities of Suspended Solids Discharged in Final Effluent.

Pertinent reported data from Table I is listed in the first three columns of the Table below. In column 4 the flow has been converted from gpd to MGD. The quantity of suspended solids is obtained by multiplying the value in column 3 (mg/l) by the value in column 4 (MGD), and then multiplying the result by the factor 3.78. This is expressed in mathematical form as:

KG/day = MGD x mg/l x 3.78

Example: On September 3, Kg/day = 0.33 x 12 x 3.78 = 15

Date	Flow gpd	T.S.S. mg/l	Flow MGD	T.S.S. Kg/day
3	326,900	12	0.33	15
6	458,500	16	0.46	28
9	146,000	25	0.146	13.8
12	519,200	25	0.52	49
16	708,900	60	0.71	161
20	272,900	19	0.27	19
23	761,800	23	0.76	66
26	291,600	20	0.29	22
29	525,600	25	0.53	50
Total		225		424

$$\text{Average T.S.S.} = \frac{225}{9} = 25.0 \text{ mg/l}$$

$$\text{Average T.S.S.} = \frac{424}{9} = 47.1 \text{ kg/day}$$

TRAINING GUIDE NOTE	REFERENCES/RESOURCES

H.1 Computation of percent suspended solids removal.

Pertinent reported data from Table I is listed in the first three columns of the Table below. The percent suspended solids removal for each day appears in column 4.

The percent removal is obtained by subtracting the concentration of suspended solids in the final effluent from that in the plant influent, dividing this difference by the concentration of suspended solids in the plant influent, and multiplying the result by 100. This can be expressed in mathematical form as follows:

$$\% \text{ T.S.S. Removal} = \frac{\text{Influent T.S.S. (mg/l) - effluent T.S.S. (mg/l)}}{\text{Influent T.S.S. (mg/l)}} \times 100$$

Example:

$$\text{On September 3, \% T.S.S. removal} = \frac{171 - 12}{171} \times 100 = 93.0\%$$

Date	T.S.S.-mg/l Inf.	T.S.S.-mg/l Eff.	% Removal
3	171	12	93.0
6	168	16	90.5
9	200	25	87.5
12	198	25	87.4
16	180	60	66.6
20	170	19	88.8
23	186	23	87.6
26	195	20	89.7
29	195	25	87.2
Total	1,663	225	
Average	185	25	

$$\text{Average \% Removal} = \frac{185-25}{185} \times 100 = 86.5\%$$

I.1 Computation of the Geometric Mean

Pertinent reported data from Table I is listed in the first two columns of the Table below. The logarithm of the reported Coliform value appears in column 3. Note that two-place logarithms are used in this calculation. That is, the mantissa of the logarithm (the numbers to the right of the decimal point) contains only two numbers. Two-place logarithms are adequate since the coliform values are reported only to two significant figures.

The logarithms in column 3 are added, the total is divided by the number of values reported, and the anti-logarithm of the quotient is obtained. This is the geometric mean. It is reported to two significant figures.

Date	Fecal Coliform N/100 ml	Log of Fecal Coliform
3	350	2.54
6	540	2.73
9	180	2.26
12	170	2.23
16	220	2.34
20	240	2.38
23	110	2.04
26	130	2.11
29	280	2.45
	Total	21.08

$\frac{21.08}{9} = 2.34$

The antilogarithm of 2.34 is 220. This is the geometric mean. If the antilogarithm did not end with a zero, the number would be rounded to the nearest ten for reporting purposes.

	TRAINING GUIDE NOTE	REFERENCES/RESOURCES
C.1.1.	Reporting of minimum and/or maximum values may or may not be required by the permit-issuing authority. If not required, a dash or an asterisk may already be entered in either or both of these spaces. The same is true for all of the other parameters shown, with the exception of pH, for which minimum and maximum values must be reported.	
C.1.3.3a	Preprinted forms may already have either a dash or an asterisk in this space. This also applies to all other cases in this procedure where the entry of a dash in a space is specified.	